Supporting Information

Live Cell-Integrated SPR Biosensing Approach to Mimic the Regulation of Angiogenic Switch (VEGF-VEGFR) upon Anti-Cancer Drug Exposure

Chang Liu[1], Subbiah Alwarappan[2], Haitham A. Badr[3], Rui Zhang[1], Hongyun Liu[4], Jun-jie Zhu[5], Chen-Zhong Li[1]*

[1] Nanobioengineering/Bioelectronics Laboratory, Department of Biomedical Engineering, Florida International University, Miami, FL 33174, USA.

[2]Bio-electrochemistry Research Group, CSIR-Central Electrochemical Research Institute, Karaikudi, 630006, Tamilnadu, India.

[3] Department of Biochemistry, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

[4] College of Chemistry, Beijing Normal University, China

[5]State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, China

Indication	Sample Size	Conclusions
Colorectal Cancer	104	Bevacizumab combined with chemotherapy was well tolerated and may be effective in increasing response rates and prolonging time to disease progression in patients with metastatic colorectal cancer
Non-small cell lung cancer	99	The addiction of bevacizumab (15 mg/kg) to carboplatin/paclitaxel chemotherapy increased time to disease progression and improved response rates in patients with advanced non-small cell lung cancer
Breast cancer	28	Bevacizumab was well tolerated and might offer some benefit in patients with solid tumors in progression
Other Cancer	12	Bevacizumab combined with chemotherapy was safe and may be effective in patients with advanced cancer

Table S1. Summary of bevacizumab clinical studies.

Cell stain and fluorescent imaging

200 μM MitoTracker Red CMXRos dye and the 1.0 mM Hoechst 33342 dye were diluted into HBSS. The concentration for MitoTracker Red CMXRos dye is 30nM; The concentration for Hoechst 33342 dye is 1.0 μg/mL. Both dyes may be combined in a single staining solution. Then a sufficient amount of labeling solution was applied to cover cells adhering to substrates followed by a 15 minutes Incubation at 37°C. The labeling solution was then removed and cells were washed twice in culture medium. Finally, labeled cells were fixed with 4% formaldehyde for 15 minutes at 37°C, followed by washes in buffer and staining with any additional counterstains.

Substrates were then removed and mounted on glass microslides with anti-fade reagent/mounting medium mixture. The specimens were observed by fluorescence microscopy (Olympus IX81, Japan) with a 20x objective. The fluorescence was imaged at Hoechst channel (nucleus stain dye, ex/em: 358/461 nm), and λex (570), λem (590 nm) for mitotracker red. A CCD camera was used to capture the signals and the images were software-merged with pseudo color. The fluorescence microscope settings were kept the same throughout the experiment with the exception of the exposure time. The images were recorded at the same exposure time for Hoechst and MitoTracker Red.

Preparation of the SPR Instrument

A BI-2000 SPR instrument (Biosensing Instrument, USA) was utilized for this study. A gold SPR chip (BK7 glass slide/5nm Cr/45nm Au) was mounted on the upper face of the BK7 prism. 3-5µL index matching fluid (World Precision Instruments, USA) was applied between the SPR chip and the prism with care so that no air bubbles were trapped at the interface. Following this, a customized PDMS microfluidic injection chamber gasket with a 5mm×1.7mm×125µm channel was clamped to the gold face of the chip. Flowing buffer for both the sample and the reference channels was provided by a dual syringe pump attached to the injection chamber.

In the SPR measurement, a 670nm laser with a 72.2° incident angle was shone on the bottom of the gold layer through the prism. The surface plasmon extinction angle was tracked and plotted using the Biosensing Instrument SPR Control Program running on a PC. The sensing surface was flushed by 50mM PBS buffer (pH 7.4) at a 150 µL/min flow rate until a stable SPR baseline was acquired. The flow rate was then reduced to 50µL/min and maintained during the whole SPR measurement.

The pseudo-first-order kinetic equations

In a biological system, the kinetics of ligate-ligand or antigen-antibody interactions can be described by equation [1].

$$G + L \overset{ka}{\leftrightarrow} GL \qquad [1]$$

In this equation, ka is the association rate constant. G is the concentration of the ligand and L is the concentration of the ligate. Kinetic data in SPR are generally obtained with the concentration of the ligate and the immobilized ligand. Under the pseudo first-order reaction conditions the binding should be described by the integrated form of the rate equation (Eq. [2]).

$$Rt = |(Req - R0)[1 - \exp(-ka \times t)] + R0 \qquad [2]$$

$$Kd = \frac{kd}{ka} \qquad [3]$$

In these equations, R0 is the initial instrument response, Rt is the response at time t, Req is the maximal response of the SPR instrument, kd is the dissociation rate constant, and Kd is the dissociation equilibrium constant. The derivation of the integrated rate equation for the SPR can then be described in equation [4].

$$Rt = \frac{Rmax[L]}{Kd+[L]}(1 - \exp(-ka[L] + kd)t) \quad [4]$$

Where Rmax is the maximum response of the immobilized ligand and [L] is the ligate concentration. Differentiating equation [4] with respect to time yields:

$$\frac{dR}{dt} = \frac{Rmax[L]}{Kd+[L]}\ (ka[L] + \ kd) \times \exp((-ka[L] + \ kd)t) \quad [5]$$

At time *t* = 0, equation can be simplified to:

$$\frac{dR}{dt} = \frac{Rmax[L]}{Kd+[L]}\ (ka[L] + \ kd) = Rmax[L]ka \quad [6]$$