Development of Molecular Interaction Assay Using Magneto-Resistance Sensor

Background
Determining protein-protein interactions such as affinity, specificity, cross-reactivity is invaluable during discovery and development of therapeutic proteins. Especially, one molecular kinetics in vivo is different from in vitro. So, the measurement technology of sample in biological fluids is required (1). We demonstrate that the assay using magneto-resistance sensor can be used to probe protein-protein interactions and measure correctly kinetics parameters including association and rate constants in biological fluids.

Results
The calculated kinetic parameters using the magneto-resistance sensor are compared with the values of SPR methods. Uniquely, the magnetic sensors provide reliable binding measurement in complicated matrices such as serum.

Methods
Measurement Protein
(i) Human TSH – commercial TSH antibody
(ii) Human VEGF - commercial VEGF antibody (Bevacizumab)

Magneto-Resistance Sensor
Proteins (TSH, VEGF) were applied to the surface of magneto-resistance sensors (MR813 System, MagArray, Inc.) at different concentrations, from which an optimal condition would be selected for kinetic studies. Each of antibodies was individually conjugated to magnetic beads. Both the sensor surface and modified magnetic beads were blocked following conventional methods to prevent non-specific interactions. After the sensors were being read, the modified magnetic beads were added, and the signals generates in real-time. The assay buffers were PBS for TSH and BSA/PBS (0.5% BlockAce, 2.5% BSA, 2.5% acidified BSA) for VEGF. Since only proximity signals were detected, they only reflected the binding between magnetic beads and the surface proteins.

Surface Plasmon Resonance (SPR)
SPR experiments were performed on a Biacore x100 instrument using a streptavidin-coated (5A) chip at 25°C. The running buffer was filtered HEPES buffered saline (10mM HEPES, 150mM NaCl, 3.4mM EDTA, 0.05% polysorbate20, pH 7.4). Kd and Ka were obtained by nonlinear regression analysis of the primary sensorgram data according to a 1:1 binding model using the Biacore x100 evaluation software provided by the manufacturer. Ka was calculated same equations.

Kinetic analysis scores

References