Developing new, efficient immunodiagnostic technologies

Researchers at the Kyoto Institute of Technology chose Tecan's microplate readers and microarray processing instruments to develop highly sensitive immunodiagnostic technologies based on immobilized single-chain variable fragments (scFvs).

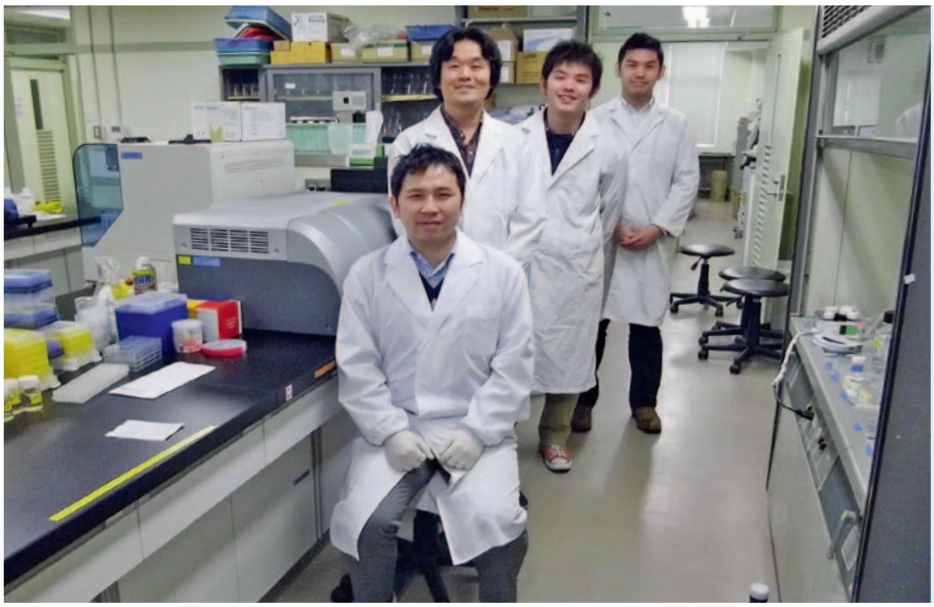
The Department of Biomolecular Engineering at the Kyoto Institute of Technology, Graduate School of Science and Technology in Kyoto, Japan, uses biochemical engineering to develop new technological applications that mimic biological functions. Dr Yoichi Kumada, Assistant Professor at the Department, is conducting research to develop new immunodiagnostic technologies based on scFvs immobilized on laboratory plasticware.

"Currently used immunodiagnostic technologies are mainly based on ELISA and bead assays, but there are technical limitations in the immobilization of the antibodies, so we are exploring ways to improve this," explained Dr Kumada. "We focused on the polystyrene in plasticware that is commonly used for routine laboratory work, and identified a peptide tag that recognizes the polystyrene substrate surface with a very strong affinity (polystyrene-affinity peptide: PS-tag). In addition, we decided to use scFvs; while normal antibodies are Y-shaped molecules consisting of a combination of four peptide chains, scFvs have just one chain. The merit of this is that they are relatively inexpensive to produce; conventional monoclonal antibodies are produced by animal cells, but recombinant antibodies that are generated by *E. coli* can be produced at a considerably reduced cost. Furthermore, they are relatively small, so they can potentially be used at a higher density, contributing to higher test sensitivity. Their molecular structure is also conducive to a more favorable alignment when immobilized. By gene recombination technologies, we designed an scFv with the PS-tag (PS-tagged scFv) and produced this using *E. coli*."

Kyoto Institute of Technology

In order to efficiently analyze and optimize the immobilization process, Dr Kumada chose Tecan's Sunrise™ and Infinite® 200 microplate readers. He continued: "We use the readers to measure the density of the PS-tagged scFvs that have been immobilized on the substrate in 96-well microplates, and to evaluate antigen binding activity. To measure density, we stain the immobilized proteins and measure their absorbance while, to measure activity, we perform an ELISA-based reaction, then measure the resulting fluorescence. Using this data, we can calculate the relative activity per molecule of the PS-tagged scFvs, and evaluate whether the antibodies can be immobilized stably and at high density using our technology. Furthermore, to measure the availability or, in other words, the exposure of the antibody, we use a fluorescence-labeled antibody that detects our immobilized scFvs, and again measure the fluorescence. We have shown that PS-tagged scFvs can be immobilized on polystyrene plates at high density, highly favorably aligned and with high activity. Compared to the conventional whole antibodies (mAb), our PS-tagged scFvs can be manufactured at less than 10 % of the cost, and have improved test sensitivities by 10 to 100 times."

Dr Kumada described the instruments' strengths: "The Sunrise and Infinite give us more reliable readings compared to other instruments that we used previously, particularly giving good resolution even at low intensities. The Sunrise comes with Magellan™ software, which has a very useful absorbance kinetics function; our measurements are often taken at 30 second intervals for 30 minutes, and Magellan automatically plots the kinetics of each well on a chart. With the Infinite, we have found



The Department of Biomolecular Engineering team at the Kyoto Institute of Technology. Front to back; Dr Yoichi Kumada, Dr Lee Joo-Young, Kagenari Yamakawa, Yusuke Emori

that the selection of the measurement area is very easy, and Tecan also provides excellent support for non-standard plates; we have only needed to make a call, and a script has been written to allow us to use multi-well plates of different formats."

More recently, the Biomolecular Engineering laboratory has started using Tecan's microarray-processing instruments to evaluate the antigen binding activity of the PS-tagged scFvs that have been immobilized on biochips. Dr Kumada continued: "We use Tecan's HS400™ Pro hybridization station for automated processing of our scFv-immobilized biochips, including blocking, washing, antigen-binding and fluorescence-labeled antibody binding. The same slide carrier used in the HS400 Pro is compatible with Tecan's LS Reloaded™ scanner, so we can easily transfer the chips to the microarray scanner for fluorescence detection. We have found the scanner to have very good reproducibility, giving us confidence in our data from quantitative experiments, and its accompanying software makes it very easy to use."

"Although DNA chips are often used these days, protein chips are somewhat behind in their development," explained Dr Kumada. "A few are available for research, but products for practical use are very limited, and I hope that the technologies that we are developing will help these products to become reality. We are continuing our research to develop immunoassays, biosensors and antibody arrays using PS-tagged antibodies, with the aim of implementing their practical applications."

To find out more about Tecan's microplate readers, visit **www.tecan.com/detection**

To find out more about the Kyoto Institute of Technology, visit **www.kit.ac.jp/english**

Antibody and the single chain variable fragment (scFv)