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Octet Systems

Expanding Screening and Ideas! High-Throughput Analysis Prominent in Antibody Drug Development

Sartorius Japan K.K.

Antibody drugs are becoming the pillars of the treatment for various diseases, including in the field of cancer research. In research and development, screening is indispensable in many processes. Chugai Pharmaceutical Co., Ltd., which developed the first antibody drug, Actemra, in the country and has the largest market share of antibody drugs in Japan, is continuing to build a unique screening system to produce and evaluate 1,000 antibody variants in about two weeks and improve screening and modification of new antibodies with technologies such as antibody engineering technology that enhances blood retention and disease-site specificity of the antibodies. In recent years, with the expectation to further accelerate the research, the company introduced Octet Systems of Sartorius AG to realize the high throughput of binding kinetics analysis.

The Octet Systems, a biomolecular interaction analysis system from Sartorius, is an automated assay system that incorporates the Dip and Read assays and Bio-Layer Interferometry (BLI) released by ForteBio in 2005 to measure and analyze the interactions between biomolecules label-free and in real-time. We interviewed Dr. Akari Kawazoe from the Discovery Biologics Department, Chugai Pharmaceutical, on how Octet is being used to provide high-speed processing and efficiency sought in the expanding antibody drug market.



Discovery Biologics Department, Chugai Pharmaceutical Co., Ltd.
Dr. Akari Kawazoe

- How are you involved in the development of antibody drugs?

I am mainly responsible for antibody screening and data acquisition on early-stage Chemistry, Manufacturing and Control (CMC) development. We acquire lead antibodies and make modifications to increase the binding capacity to antigens, facilitate stable handling in the process of manufacturing as pharmaceuticals, or enhance ease of use when they reach hospitals and patients. In the case of antibody drugs, amino acid modifications are performed. However, even if you change one amino acid, the properties of the antibody will change greatly, and even if the binding activity increases, the stability during storage may deteriorate. So, by considering a wide range of parameters, we produce candidates of antibody drugs with the most balanced combination of sequences with enhanced activity and stability. I comprehensively acquire data and evaluate the binding capacity and storage stability in this process.

I have had a lot of experience in physical and chemical analysis experiments using instruments such as HPLC, but since 2012, I have also been in charge of interaction analysis, and now I am the group manager for the protein analysis group. From 2012 to 2015, I was involved in the startup of Chugai Pharmabody Research Pte. Ltd. in Singapore where I gained experience in the startup of screening systems using Octet.

Encourage New Experiment and Realize Speed Upgrade

- How was Octet introduced to Chugai Pharmaceutical?

Around 2011, a team working on animal immunity acquiring lead antibodies first introduced Octet. A wide variety of antibodies can be produced when animals are immunized and antibodies are made in vivo, but in order to judge their efficacy and advance them to the next step, there was a need to be able to easily characterize and distinguish as many promising ones as possible, so it was important to measure them with good throughput. In addition, samples taken from animals are often used in screening as supernatant from cultured cells and contain impurity proteins other than antibodies. Since Octet immerses the biosensors in the sample prepared in the measurement plate to perform the measurement, the sample does not flow into the instrument. Therefore, there is no intricacy to perform maintenance every time the measurement is performed, and there is only a small risk of failure due to clogged or dirty flow channels. Sensors are low-priced so it is suitable for single use, and it is also an advantage to be able to skip the evaluation of conditions for the stable use of the sensors. We have noticed that they are a very nice match to the mass screening of samples with a low degree of purification.

- It looks like it was useful for the in-house research teams that value high throughput. How was the use of Octet expanded for your protein analysis function?

Since around 2015, the number of samples and the time spent evaluating them have dramatically changed. From around this time, various technologies have advanced and the designing of variants that took about two weeks has become possible to be done in about a week, and data acquisition of hundreds of samples have been requested in parallel from multiple projects. Previously, we were carefully measuring about 100 samples per week using SPR, but the situation changed such that we were getting asked to evaluate the same number of samples in a few days. We had to keep up with the pace of changes in the needs and speed of other functions, so we investigated the active use of high-throughput instruments. As a result of implementing Octet while considering whether similar KDs can be obtained compared to conventional data, similar experiments can be designed, and rankings can be done with the same range of interaction, it was possible to respond to changes in the speed and needs of the projects by using it for screening.

- What are the benefits of using Octet?

I feel that the speed of experiments has gone up because Octet can be used for speculation ahead of time. For example, when we decide to perform a new experiment in binding kinetics analysis, we may try some initial conditions to see whether the design will work. Octet is intuitive to operate so it is handy and easy to use. There is also a large variety of ready-to-use biosensors, and even if we fail, there is low risk in terms of running cost since we can throw them away after each use. I have an impression that there are many users who can freely work on experiments, saying, "I don't know how it will work, but I will try it."

In addition, in SPR, proteins are nonspecifically adsorbed due to the influence of dextran and the gold film on the surface of sensor chips, making them difficult to visualize the meaningful bonds. With the Octet biosensors, however, they can often be measured without the nonspecific adsorption. In cases where SPR is used for proteins with electrical charge imbalance, the proteins can become too adsorbed to the surface and the apparent bonds can be stronger, or the binding can be very slow due to charge repulsion, leading to weaker apparent bonds than they

actually are. Using Octet, these data may be retrieved more neatly. If the measurement target seems to be incompatible with the conventional method, I try it with Octet.

Taking advantage of the real-time observation feature, we may be able to recognize the points that should be corrected in other experiments. For example, we examine multi-step reactions by overlapping primary and secondary antibodies in binding evaluation using ELISA, but it is not possible to identify at what step it did not fluoresce in a single experiment result. If this is reproduced with Octet, it is possible to follow this over time, such as whether it was at the point captured by the biosensors or at the next step, making it possible to visualize where needs a fix.

I think it is also a large advantage that there are a large variety of biosensors. Since it is different which biosensors can capture most stably and how much more can be captured at the same concentration, I would like to choose a sensor that can produce the necessary sensitivity. Having a large variety is also important to confirm detailed systems because we can select an appropriate sensor by distinguishing the orientation of antibodies to be captured. I always stock at least one bag of ProA, ProG, and ProL,



AHC and FAB2G for human antibodies, and AMC for mouse antibodies so that I can quickly get to work. Also, since 1 ligand x 1 analyte reaction can be independently measured with a single sensor, this allows for different types of sensors to be mixed in a single measurement. It is possible to immobilize ligands in individual flow cells with SPR, but it takes time and effort and it is difficult to simultaneously measure them all at once when the conditions of regeneration are different, so that is why the Octet's features shine.

Octet's very high throughput and extensibility have set an environment in 2012 that allowed ten 96-well plates, approximately 1,000 samples, to be measured continuously in a single screening of animal immunity in upstream processing. By replacing the plates with autonomous operation and processing overnight, the first stage of screening to start antibody engineering can ensure an ample capacity to accept 2,000 measurements per week. Early screening is not difficult because biosensors are single-use, but when it comes to measuring many cycles while regenerating, it will be difficult to design as the complex movements must be controlled.

Automation Is the Key to Create New Concepts

- What will be necessary to further accelerate research in the future?

There are many voices within the company wishing for more freedom in software. Octet's use is by far the most intuitive, and the number of users and samples have increased as it is easier to use, growing the request to try various measurements and analyses. In fact, Octet is used not only in upstream processes, but also in midstream and downstream processes with higher complexity. When conducting new trials, I feel that if the degree of freedom not limited by general intermolecular interaction analysis in software increases, more complex measurements and analyses that were previously difficult to perform will be made possible, leading to facilitate more research.

I also wish that robots that can automatically prepare samples and arrange plates can be put on sale along with Octet. We are constantly working on developing new technology, which is rapidly increasing the data that we wish to collect. To create an unprecedented concept, we need to evaluate 10 times or 100 times the amount of samples, and there is an increasing need to make the process of creating what is necessary as easy as possible. The huge increase in the tasks is the status quo, including distinguishing what instrument to use and adjusting the parameter settings depending on each project, instead of repeating

measurements in templates. If automation by robots is made possible, samples can always be prepared, and it can be made possible to immediately measure with Octet. I want to leave what can be done fully automatically to robots to some extent, so that people can focus on using their brains. Octet automation is drastically advanced among the analysis equipment we use in our company. Since there are some devices that operate by assembling a large system that even connects a sample cooler, a centrifugal machine, and a measurement instrument, I have a feeling that it will be possible to process 24 hours a day in the future.

- Will the roles of researchers change as AI and automation develop?

To improve the accuracy of AI and machine learning, we would need a huge amount of teaching data and algorithms that accurately classify those data, but we, as humans, are able to gain insights from limited information and experience so that we can avoid troubles, and I think this is our unique feature. While deep learning may make certain things possible, researchers are able to use their expertise and past experience to connect seemingly unrelated matters. Sometimes, different areas of expertise are connected to create extraordinary ideas. We have a corporate culture where researchers feel free to interact with each other and try out new ideas, saying, "let's try it, it sounds interesting." Currently, due to the influence of COVID-19, I feel that there are fewer opportunities that spark new ideas from chatting with each other within the company, but I think it is a unique feature of humans to generate ideas from fortuitous encounters. Octet is very suitable for people who want to carry out such casual ideas and thoughts or experiments. As long as the material for the experiment is available, it will hopefully take just about an hour to get the results, so it encourages the casual trials. With the conventional method, it takes a day no matter how hard you try, and we feel devastated when it does not work out. I feel that Octet is very useful as it supports inspiration, so I can stay that the breadth and opportunities of research have increased considerably.

When we didn't have the choice of methods, we might have been bound by the mind of "we have to do it that way." In the field, even if there is a gold standard, I think it is essential to have a sense that the standard is correct when we actually use it. What do we need for our experiment? Can we ascertain what we are actually after? As the basic attitude as researchers, the entire team must judge the introduction of instruments and experiments based on solid experience, not because "this is the only instrument available," or "this is the standard that everyone follows." I am very grateful that there is a foundation in the company that we can receive recommendations from users who have experience in such cases.

Delivering Antibody Drugs Faster and Cheaper

- The development of antibody drugs will be the key to various disease treatments in the future. How will you tackle this as a researcher?

The antibody drugs currently used as products are the results of projects that were conducted in nonclinical research about 15 years ago. If Octet existed at the time, the quality would not change, but we could have made them available earlier. Our mission is to deliver effective therapeutics as soon as possible at low prices, and we always wish that the drugs we developed as researchers will save patients. Sometimes, however, we tend to forget this. We have had patients write a letter of gratitude to the

company, and it is shared with the research members involved in the development, which is very nice and rewarding working in the field. The company also creates opportunities to listen to medical personnel and patients who have used our therapeutics at general meetings. When we actually listen to their stories, everyone's eyes sparkle and it gives us motivation and empowers us to work even harder.

There is still room for improvement and growth in technology, and I am confident that there are more drugs that can be developed. Since my job is to realize new ideas, I think that I can make a large contribution to the world if I can use Octet to try various analyses in combination with automation and digitalization to improve throughput.



Octet R2



Octet R4



Octet R8



Octet RED384



Octet HTX

Octet Systems for Biomolecular Interaction Analysis

(Interview/Article by Nozomi Sakamoto)

Sartorius Japan K.K.
Simplifying Progress

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The laboratory products and service divisions of Sartorius meet the needs of researchers and technicians engaged in R&D and quality control in the medical and various fields by providing innovative laboratory equipment and consumables.

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