SARTORIUS

Expert Interview

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Biolayer Interferometry Enables Fast Interaction Screening for Structural Biology

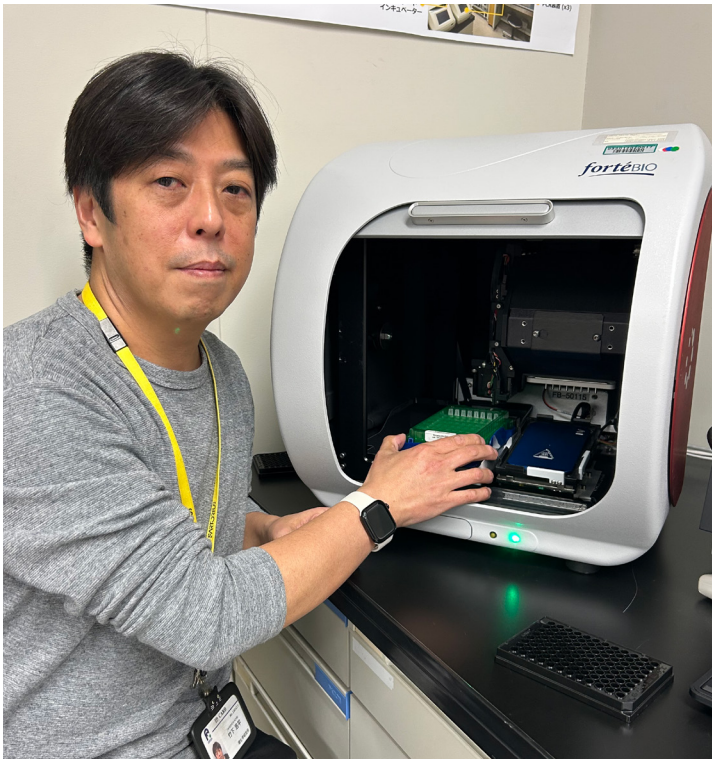
Introduction

As structural biology advances, its potential in drug discovery is growing. Recent reviews show that cryo-EM plus AI structure prediction yields high-resolution, druggable models for ligand design and optimization.

High-quality, high-throughput interaction analysis is essential to these workflows. By rapidly characterizing biomolecular interactions, researchers can select and qualify the best proteins, antibodies, and complexes before investing in crystallography, cryo-electron microscopy (cryo-EM), or nuclear magnetic resonance (NMR).

Dr. Kohei Takeshita, a structural biologist and research scientist at the RIKEN Spring-8 Center, has made important contributions to structural analysis, including work on supramolecular complex or membrane proteins. In 2018, he began using the Octet® Bio-Layer Interferometry (BLI) system in his CRISPR-Cas3 research to measure and confirm interactions between the Cascade complex, which includes CRISPR RNA (crRNA), and target DNA. These studies helped clarify how CRISPR-Cas3 cleaves double-stranded DNA.

We spoke with Dr. Takeshita about how Octet® BLI fits into his protein science and structural biology workflow.



Dr. Kohei Takeshita with Octet® RED96
(currently Octet® R8, and the more advanced Octet® R8e)

Could you tell us a little about the research you do?

I work in protein science and structural biology, with a current focus on proteins related to genome science, ion channels, and enzymes. Understanding the atomic-level structure of proteins tells us about their roles and mechanisms, and we can use this information in drug discovery applications, for example, to modify molecular function.

What inspired you to work on CRISPR-Cas3, and how has your research in this field evolved?

CRISPR-Cas3 was developed as a genome editing tool by Professor Tomoji Mashimo and Associate Professor Kazuto Yoshimi from the Division of Animal Genetics at the Graduate School of Frontier Sciences, The University of Tokyo. We met at Osaka University. Professor Mashimo approached me about producing high-quality CRISPR-Cas3 proteins for genome editing in cells, and we started working together. Our group focuses on structural biology to produce and modify proteins for CRISPR-Cas3.

What led you to use the Octet® BLI system for biomolecular interaction analysis, and how has it impacted your work?

Structural biology research involves specialized instruments such as X-ray crystallography NMR, high-speed AFM, and cryo-EM. To get the best results with these tools, we must have high-quality proteins. We produce recombinant proteins from *E. coli*, yeast, insects, and animal cells, and refine them to the highest purity.

We also assemble supramolecular complexes that function *in vivo* and protein–compound complexes for drug discovery. These require *in vitro* mixing and preparation. Before moving to structural instruments, we check that the components bind correctly and measure binding strength and how long interactions last. We use Octet® BLI for this step.

At first, I considered surface plasmon resonance (SPR), but I personally cannot find the time for the preparation and maintenance requirements. Octet® BLI was introduced in Japan around 2010 and was often described as easy to use. One was available at the RIKEN SPring-8 Center, so I tried it and, with online support during the COVID-19 period, became proficient. Octet® is indeed easy to use.

How do you use Octet® when working with antibody Fab fragments in structural studies?

Proteins are sometimes prepared in complex with antibody Fab fragments. This can improve crystallization for structural analysis and serve as markers for increased molecular weight and orientation in cryo-electron microscopy. We use Octet® early in hybridoma production to screen culture supernatants in 96-well plates and identify the best Fab candidates.

From your perspective, what makes Octet® BLI user-friendly?

There are many ways to analyze biophysical interactions. SPR is common, but it requires specialized skill because samples must run through microfluidic channels. SPR instruments are usually maintained by a manager, and there is hesitation to give wide access.

With Octet® BLI, on the other hand, the sensor is dipped directly into the sample. It is maintenance-free, ready to use after it is turned on, and the biosensors are single-use. Anyone familiar with the basic technology can operate it. I was fortunate to have access to one at my workplace.

Later, Sartorius technical specialists visited my lab to train new Octet® users. For us, the support was just as important as having an instrument that's easy to use. Even though we were not using the latest model, we still received excellent customer support.

What outcomes have you obtained using Octet® in your CRISPR-Cas3 research?

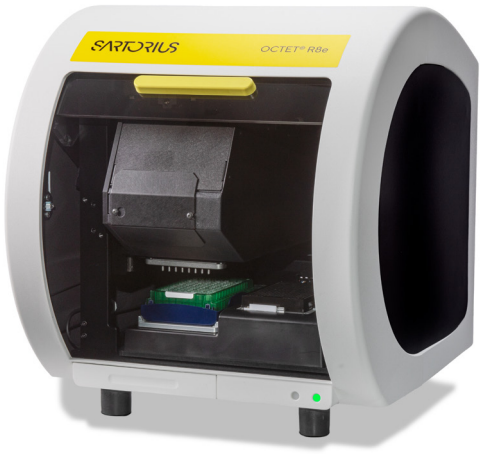
In our CRISPR-Cas³ work, we focused on the Cascade complex, which carries CRISPR RNA (crRNA) to the target DNA. We first measured how strongly Cascade binds single-stranded target DNA (TS) versus non-target DNA (NTS) and looked at how PAM sequences affected those affinities across six conditions.

We then did the same with double-stranded DNA, comparing target and non-target strands and again testing PAM-dependent differences across seven conditions.

Finally, we compared binding to DNA that perfectly matched the crRNA with partially matched and non-matched DNA, across seven conditions. Taken together, these assays clarified how Cascade recognizes and binds its target DNA, and we published the results.

Further Reading

Yoshimi K, Takeshita K, Kodera N, Shibumura S, Yamauchi Y, Omatsu M, Umeda K, Kunihiro Y, Yamamoto M, Mashimo T. Dynamic mechanisms of CRISPR interference by Escherichia coli CRISPR-Cas3. Nature Communications 13(1), 2022.



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