A Therapeutic Antibody Characterization Trinity Accelerates Drug Development

"Antibodies with unique epitopes that may offer mechanistically differentiated modes of action and intellectual property opportunities are highly desirable as therapeutics," explains Yasmina Noubia Abdiche, PhD, chief science officer, Carterra. "This makes screening by epitope more relevant than screening by affinity, since affinity can be optimized by standard protein engineering. Furthermore, since an antibody's epitope is innate and cannot be predicted or designed rationally by in silico methods, it must be selected empirically."

Epitope binning assays can be used to assess the epitope diversity of an antibody library, by testing antibodies for their ability to block one another's binding to their specific antigen and thereby clustering them into epitope families or bins. Since bin members likely share functional characteristics, bin representatives can be chosen to distill the panel to a subset for further study, while retaining the epitope diversity of the whole panel.

Making informed decisions faster

Different animal species recognize antigens differently, and thus produce different antibodies. To leverage this diversity, Ligand Pharmaceuticals develops novel transgenic animal antibody discovery platforms. The company's OmniAb® (Ligand Pharmaceuticals) currently comprises five distinct platforms for generating fully human antibodies, OmniMouse®, OmniRat®, OmniFlic®, OmniChicken®, and OmniClic®.

"Since chickens are phylogenetically distant from mammals, their proteins share less sequence homology with those of humans, so human proteins are often strongly immunogenic and can readily elicit rodent cross-reactive or pan-mammalian antibodies," points out Bill Harriman, PhD, vice president, Antibody Discovery Services, Ligand Pharmaceuticals. "These cross-reactive antibodies are very useful when studying the antibody's physiological effect in preclinical animal disease models and to confirm mechanism of action."

Ligand Pharmaceuticals collaborated with Carterra to generate data on two model antigens, and found that the binding properties of antibodies, produced in wild-type (WT) chickens and mice differed in some therapeutically advantageous ways. These results showed that the epitope coverage produced in chickens overlapped with and extended beyond that available in mice because the chicken produced some antibodies that were cross-reactive to the mouse version of the model antigens studied.

For one of the targets, the immunizations were repeated using the OmniChicken platform to generate a new panel of antibodies. This panel recapitulated the epitope coverage observed from the WT chickens, validating the OmniChicken platform. As the company expands its portfolio, benchmarking the epitope coverage produced by any new engineered variant against WT provides essential validating data.

"Carterra's LSA™ high-throughput surface plasmon resonance (SPR) instrument is an excellent tool to monitor new genotypes and to generate data to demonstrate that the transgenic animals are immunologically robust," notes Harriman. "ELISAs provided only a crude binary measure, whereas epitope binning data produced on the LSA gives us a detailed picture of the epitope landscape of our antibody libraries quickly using crude samples."

"The three core applications of high throughput SPR that form the antibody characterization trinity are capture kinetics, epitope binning, and epitope mapping. Together, they provide a comprehensive characterization of antibody libraries with minimal sample consumption, enabling more confident decisions to be made earlier and obviating the need for preliminary ELISA screening," confirms Abdiche.

Full kinetic analyses and epitope characterization

As the number of companies performing antibody discovery increases, the need for early screening of candidates is essential to those companies that intend to be competitive. LakePharma, a leading U.S.-based biologics company, specializes in antibody discovery, antibody engineering, protein chemistry, bioexpression, bioprocessing, bioanalytics, and biomanufacturing services.

"Antibody screening, characterization, and epitope binning is a critical part of our therapeutic antibody discovery and development process," says Raphael Levy, PhD, director, Antibody Engineering, LakePharma.

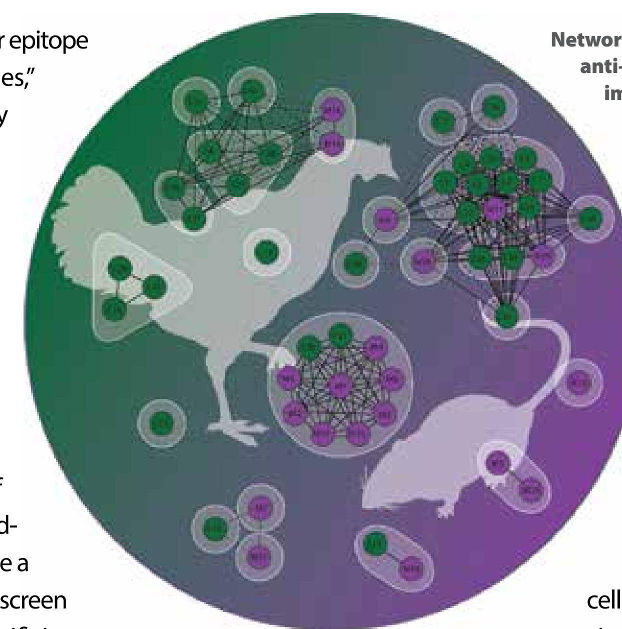
"When we used only a BLI-based (biolayer interferometry) Octet system, we were unable to perform full high-throughput kinetic analysis of unpurified or partially purified extracts or epitope binning on a large number of antibodies," he continues. "The Carterra technology changed that and gave us the ability to perform very sensitive high-throughput full-kinetic analysis of unpurified bacterial extracts."

The LSA™ high-throughput surface plasmon resonance (SPR) instrument is a fully integrated antibody screening and characterization platform that can analyze up to 384 binding interactions simultaneously, delivering 100× the data in 10% of the time with 1% of the sample requirements of other systems.

By employing a one-on-many assay format, samples are analyzed in a highly parallel style, significantly accelerating throughput while conserving precious samples. Unattended, the LSA can perform a full kinetic analysis of a specific target binding to 1152 antibodies in a capture kinetics format; complete a full 384 × 384 epitope binning assay; or screen >384 antibodies for their binding specificity to a 384-peptide array to determine epitope mapping.

"Having a platform that can give you not just specificity but also binding kinetics and that allows you to rank clones at a stage where you only have small volumes is very amenable to high-throughput antibody discovery," insists Harriman.

The results of a binning experiment are represented graphically, such as via proprietary network plots, in which blocking relationships between antibodies are indicated with cords, and bins are inscribed by envelopes. "Merging data from independent assays into the networks and coloring them accordingly provides a strikingly intuitive visualization tool for organizing multiparameter information, facilitating the discrimination of clones with unique behaviors," adds Abdiche.



Networks showing the epitope coverage of anti-progranulin antibodies sourced from the immunization of mouse (purple) or chicken (green), as determined by high throughput epitope binning assays. The cords represent the blocking relationships between the antibodies (nodes) and the envelopes inscribe the bins.

Facilitating high-throughput antibody discovery

Reproducibility, a persistent problem in science, is partially due to poor-quality reagents. The Institute for Protein Innovation (IPI), the vision and brainchild of Timothy Springer, PhD, a Harvard Medical School professor of medicine, seeks to remedy this. The not-for-profit entity's central mission is the antibody initiative that plans to generate open-source, high-quality recombinant antibodies against all human and mouse cell surface proteins.

"We will be honest and open about our endeavor so scientists will be able to draw rational conclusions about our work," discusses James Love, PhD, chief operations officer, IPI. "Importantly, the synthetic recombinant antibodies that we plan to make will be an immortal resource for biomedical research.

"Our plan is to develop multiple antibodies against each of the cell surface proteins. Since there are about 5000–6000 human cell surface and secreted proteins, that means we could be producing tens of thousands of new antibodies. A key part is to look at how well these antibodies bind, to rank them kinetically, to check specificity, and to perform epitope binning to define their binding epitopes."

"The LSA has the throughput to test large numbers of antibodies quickly," Love indicates, "and is really good at epitope binning, which other systems do not seem to do as well."

To make the soluble domain of a human cell surface protein, the synthetic DNA of the protein is expressed in human embryonic kidney (HEK) cells, and the excreted protein purified. Next, is a yeast cell surface selection method.

IPI has built a large library of Fab fragments, billions of transformants that are tethered to the surface of a yeast cell; each yeast cell encodes a different Fab fragment. A series of magnetic-activated cell sorting (MACS) and fluorescence-activated cell sorting (FACS) screens identifies the yeast that binds very tightly and specifically to the antigen. Since the Fab sequences are known, they can then be reformatted into a full immunoglobulin G molecule, or another format IPI chooses.

The LSA expedites the determination of binding specificity, kinetics, and affinity of hundreds of antigen/antibody interactions, so that the 2–3-month-long overall process converges upon a set of validated antibodies.

"Since animals are not used in our recombinant approach, highly conserved epitopes can be targeted, and the process can be turned around faster than traditional hybridoma technologies," concludes Dr. Love.

Carterra's LSA enables the rapid characterization of large panels of antibodies in terms of their binding kinetics, affinities, and epitope specificities with minimal sample consumption. Knowing the detailed binding properties of antibodies at the earliest stages of research is highly appealing to the drug discovery industry because it helps to accelerate library-to-lead triage, ultimately cutting costs and saving time in progressing leads to the clinic. ■