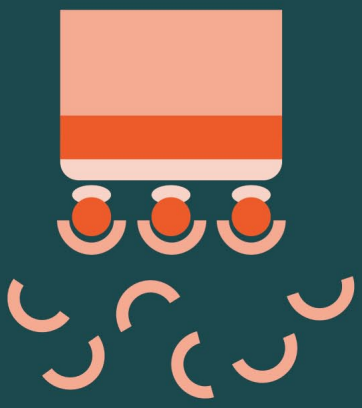
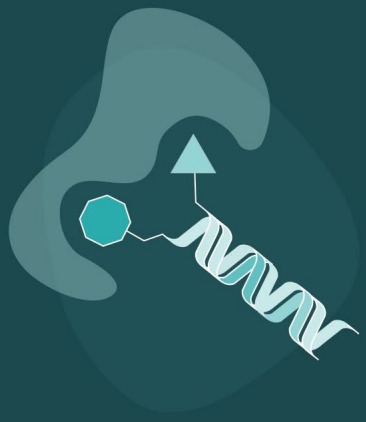
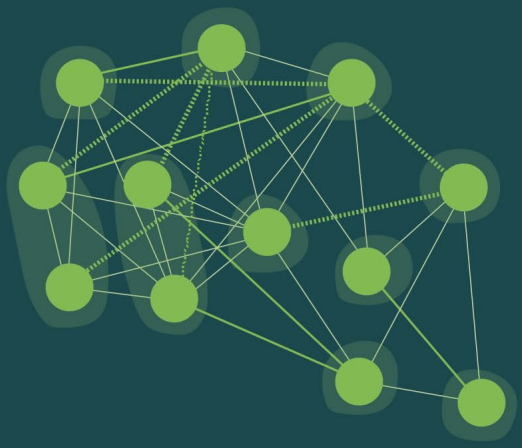
Next-Gen BLI Technology

Accelerating Antibody Discovery







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Advancing Biosensor Technology

Next-Gen Biolayer Interferometry

Next-gen biosensors to accelerate your antibody discovery

Intuitive software for quantitation, kinetics, and epitope binning

Systems designed with productivity in mind

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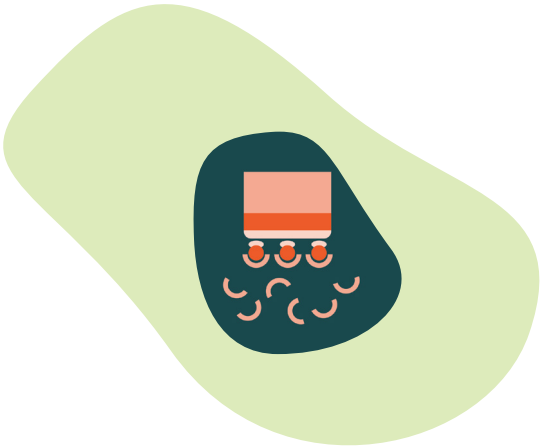

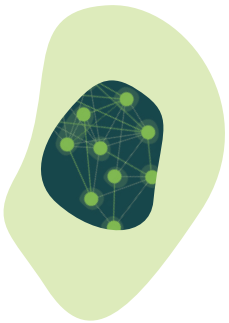

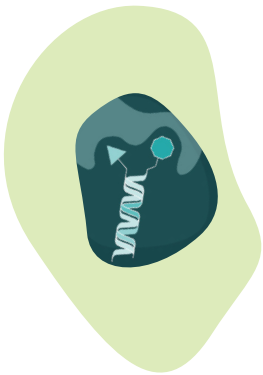

Introduction

The development of antibodies for diagnostics or therapeutics requires comprehensive characterization of affinity, specificity, and mechanism of action (https://doi.org/10.1016/j.xpro.2021.100836). Affinity measurements screen different isolates of an antibody to identify those that are most effective at binding antigen. Also, measurements of nonspecific binding to compounds that are structurally related to the antigen of interest can help establish the likelihood of cross reactivity with other molecules that accompany the antigen.

Biolayer interferometry (BLI) is a label free technique that measures the interference pattern of white light reflected from the surface of a biosensor, which indicates the presence of biomolecular interactions. The binding between a ligand immobilized on the biosensor tip and an analyte in solution produces an increase in optical thickness at the biosensor tip, resulting in a wavelength shift proportional to the extent of binding (Azmiri and Lee, 2015; Mechaly et al., 2016). The sensor tips collect readings in real time, while immersed in the analyte solution, without the need for continuous flow fluidics (Yang et al., 2017). BLI technology is widely used for quantitation of antibodies, which is fundamental to biological research and production processes. The technology is also extensively used for kinetics measurements of antibodies and small molecules to assess the strength and speed of binding of an antibody to a target. This is possible due to BLI's robustness to complex matrices, speed, accuracy, and ease of use.

Biosensors with different ligands that bind antibodies, proteins and small molecules such as streptavidin, human Fc, mouse Fc, protein A, anti-his, and Ni-NTA are routinely used. However, many of the first generation BLI biosensors have limited dynamic range, poor small molecule binding, and higher costs due to single use operation. The wider adoption of this simple and yet powerful technology was also limited by relatively expensive instrumentation and complex software.

This e-Book explores advances in biosensor technology and instrumentation that can significantly accelerate antibody discovery through efficient quantitation, kinetics, and epitope binning.

GEN Genetic Engineering & Biotechnology News

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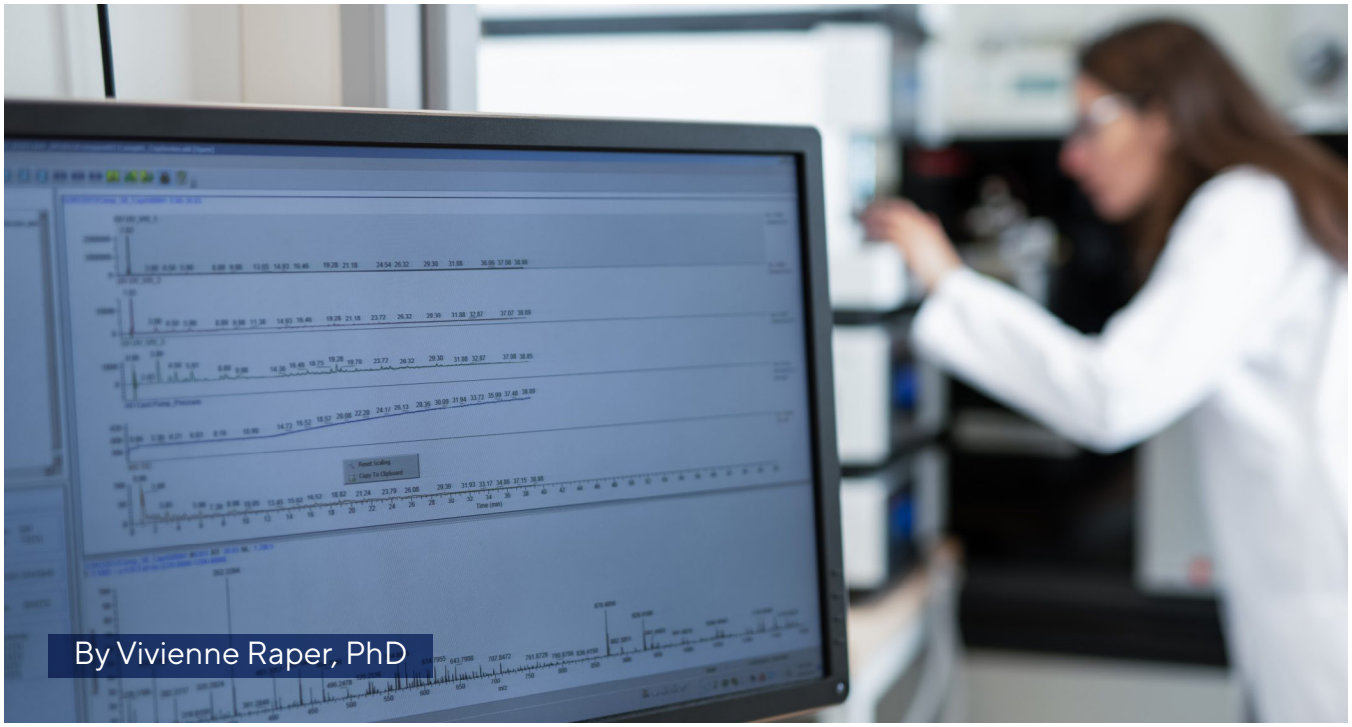
Tackling Antibody-Drug Conjugate Manufacturing Challenges



Liana Tsiatsiani, PhD, at Byondis, uses one of the company's mass spectrometers.

Antibody-Drug Conjugates (ADCs) are an exciting and emerging class of biopharmaceuticals with applications in cancer. GEN talks to Yunus Saricay, PhD, an R&D specialist at Byondis B.V., about the challenges of characterizing ADC products.

Why is ADC product characterization challenging?

ADC molecules have a complex structure with a number of different species [of molecule], each with different physical properties. Since ADCs are highly diverse molecules, you need many types of analytics to detect small changes in physical properties that can affect product quality and safety.

How is Byondis helping to overcome these challenges?

We use a multi-level analytical approach where we analyze the product from different angles to gain a comprehensive overview. We're also creative as a company, which is something I love because it generates a good environment to solve problems.

What was new about your talk at Bioprocessing Summit Europe?

There were two important novelties. The first was I highlighted how computational tools can assist with product development and analytics. I also gave several examples of how to use a structural

mass spectrometry (MS) technique, such as limited-proteolysis-based MS, to get more in-depth structural data than with conventional (low-resolution) spectroscopic methods.

ADC molecules have a complex structure with a number of different species [of molecule], each with different physical properties.

What's the role of structural analysis in helping characterize these new pharmaceuticals?

In my talk at Bioprocess Summit Europe in March, I showed that, with fluorescence

Yunus Saricay, PhD, R&D specialist at Byondis B.V.

and circular dichroism spectroscopy, a linker-drug can contribute analytical signal. Thus, you can't be sure whether the analytical signal is from the antibody, the linker-drug, or a combination of the two. Also, linker-drug conjugation can affect the sensitivity of the detection method.

So, what do you do? To confirm the structural data, we use a combination of two or three spectroscopic (low-resolution) techniques, one of these providing a mid-resolution mass spectrometry. With these methods, we can analyze structural change at the peptide level, thereby improving the quality of our structural data.

Gator® SMAP: The Power of Next-Gen BLI

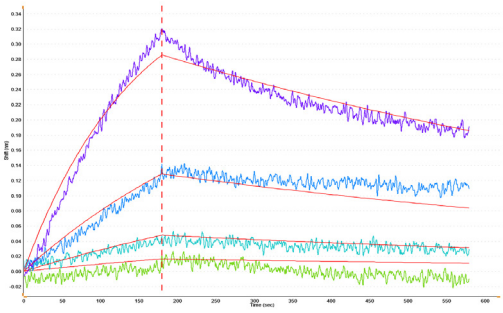
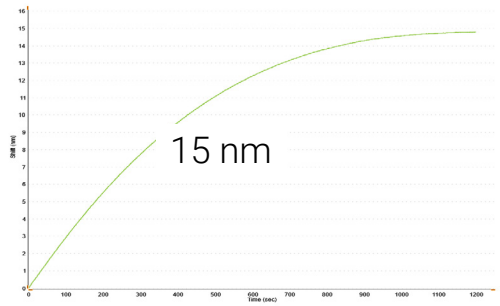
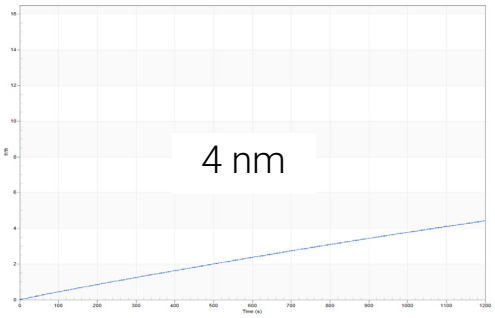
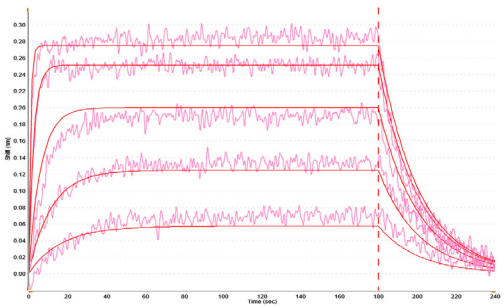
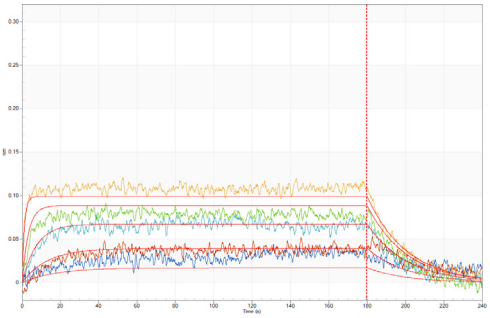
INTRODUCTION

Gator Bio has launched the SMAP (Small Molecule Analysis Probes; PN: 160011). Like other Gator Bio biosensors, SMAP has fundamental physical and chemical design elements that provide much higher sensitivity. This biosensor is sensitive enough to capture small molecules (down to 150 Da), but can also be used for biomolecules and antibodies when sensitivity is a must.

Compatible with our Gator® Prime and Gator® Plus instruments, SMAP biosensors bring a whole new level of sensitivity to biolayer interferometry (BLI) technology.

BENEFITS OF SMAP

- Higher sensitivity
- More signal
- Compatible with small molecules and biomolecules of a wide range of sizes

Gator® Platform		Other BLI Platform
	332 Da	
	Protein 52 kDa	
	330 Da	

Multi-Specific Ab Analytical Strategies

By Vivienne Raper, PhD

Credit: Morsa Images/Getty Images

A U.S. biotech company outlined its strategy to GEN for bringing multi-specific antibody therapies to market. Ichnos Sciences, headquartered in New York with facilities in Switzerland, is developing antibodies that can bind to multiple different targets for treating cancer.

"We started with a good understanding of monoclonal antibodies (mAbs) and evolved our [technology] platform to advance these complex molecules," explains Sabrina Vollers, PhD, senior team leader and head of functional and pharmacokinetic bioanalytics.

Vollers gave a talk at the annual Bioprocessing Summit Europe last month on manufacturing challenges and analytical solutions for multi-specific antibodies.

"Our success comes down to a global strategy," she explains. "On the bioanalytical side, key to the strategy is working out which assays are needed upfront and, which are needed further along as the project matures."

Method bias is a major challenge that adopting a global strategy can help overcome, continues Vollers. This is where the signal readout from an assay is complicated by target expression levels in cells and/or affinities of the multiple parts of the multi-specific antibody.

"It's not necessarily a roadblock, but it's something to be aware of," she says. "With a developability and risk assessment, you need to know early what you need to do."

According to Vollers, the company's strategy also

According to Vollers, the company's strategy also focuses on using their technology platform to tune the Fc effector function of multi-specific antibodies...

focuses on using their technology platform to tune the Fc effector function of multi-specific antibodies.

"If you have a platform-based approach, it saves a lot of development time," she tells GEN. "It's not necessarily plug and play, but you can focus on more complex function-based assays."

Another prong of their strategy is ensuring their assays have high sensitivity. As multi-specific antibodies often have more than one mechanism of action and can be more potent than mAbs, they are more likely to be formulated at low concentrations, which presents method-specific challenges, points out Vollers, adding that the company keeps all of its development and manufacturing under one roof and share information between teams.

"We keep in the mode of continuous improvement," she says. "We look at every project as a lesson learned. We're constantly streamlining our workflows and making cross-functional developability assessments. That keeps our company agile."

Another prong of Ichnos' strategy is ensuring their assays have high sensitivity. As multi-specific antibodies often have more than one mechanism of action and can be more potent than mAbs, they are more likely to be formulated at low concentrations, which presents method-specific challenges, points out Vollers, adding that the company keeps all of its development and manufacturing under one roof and share information between teams. Learn more here: **https://www.gatorbio.com/resources/publications/**

Anti-Mouse Fc Probe

INTRODUCTION

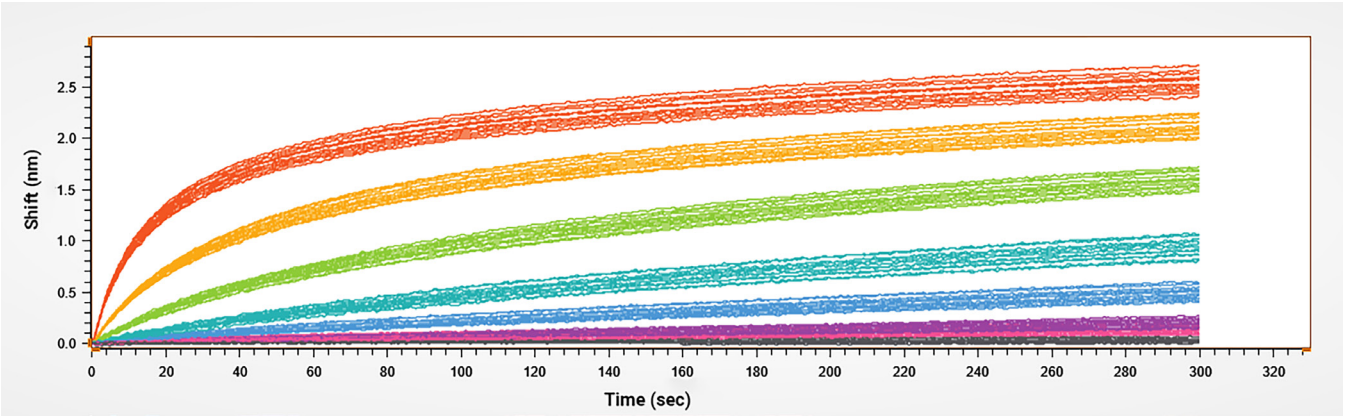
Biolayer interferometry (BLI) has been widely accepted for antibody screening, but anti-mouse Fc, one of the most widely used biosensors in mouse IgG quantitation, has remained a step behind in performance standards. Gator Bio's next generation BLI systems with novel anti-mouse Fc biosensors surpass traditional BLI's limitations with an expanded dynamic range for faster antibody screening. This next gen BLI-based high sensitivity assay improves workflow while producing fast results with significant cost savings.

COMPETITIVE PERFORMANCE COMPARISON

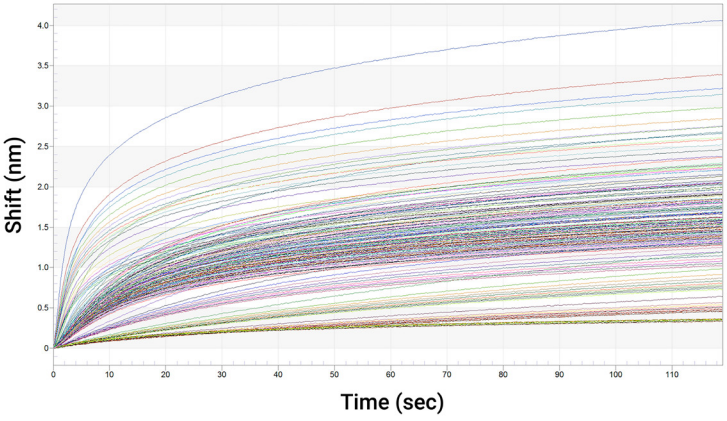
ACCURACY AND REPRODUCIBILITY AFTER 10 REGENERATIONS AT LOW CONCENTRATIONS

Gator Bio's Anti-Mouse Fc probe performance was compared with another BLI platform probe using mouse IgG standards. The quantitation was performed in the range of 0:01µg/mL – 10µg/mL using Gator® Prime and the other BLI platform. The calibration curve was created using a serial dilution of the mouse IgG standard.

Gator® BLI Platform



Other BLI Platform



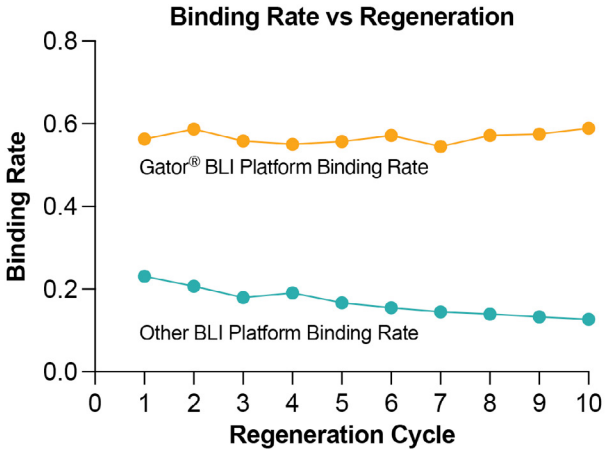
The figure at the left shows the reproducibility after 10 regenerations at 7 concentration points using Gator® Anti-Mouse Fc and probes from the other BLI platform. The Gator Bio probes show significantly clearer separation at each concentration point.

Known Conc (mg/mL)	Calculated Concentration (mg/mL)		%CV (n=10)	
	Other BLI Platform	Gator® BLI Platform	Other BLI Platform	Gator® BLI Platform
10.00	10.70	9.36	19.90	4.50
3.33	3.20	3.60	32.40	2.50
1.11	1.30	1.03	19.30	5.20
0.37	0.43	0.42	17.01	4.90
0.12	0.13	0.12	84.00	5.70
0.04	-	0.03	-	13.80
0.01	-	0.01	-	49.77

The table at the left compares accuracy and reproducibility (percent coefficient of variation, %CV) of calculated concentration using probes from the Gator® BLI platform and from the other BLI platform. While the other BLI platform is able to generate comparable accuracy at higher concentration points, the %CV is significantly higher than Gator® probes. Gator Bio delivers CV's well below 10% at almost all concentration points. Also, the other BLI platform was unable to determine concentration below 0.12 µg/mL.

Binding rate and quantitation accuracy for over 10 regenerations at 1,000 µg/mL

The binding rate and quantitation accuracy was determined using both probes at a concentration of 1,000 µg/mL for over 10 regenerations.

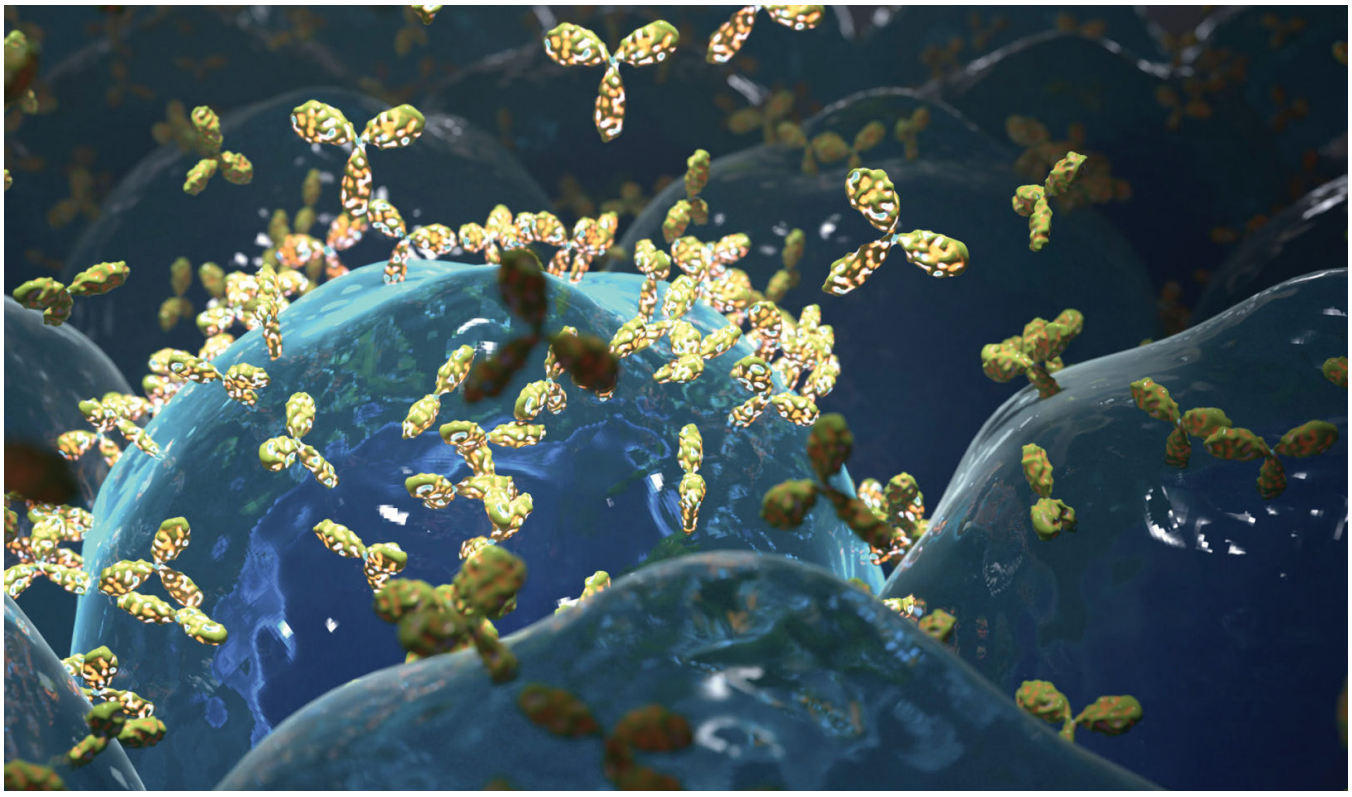


The graph at the left shows a very stable binding rate for over 10 regenerations using Gator® probes whereas the binding rate continuously drops using the other BLI platform.

Cycles	Binding Rate Comparison	
	Other BLI Platform	Gator® BLI Platform
1	0.2308	0.5630
2	0.2069	0.5870
3	0.1908	0.5580
4	0.1798	0.5500
5	0.1670	0.5570
6	0.1547	0.5720
7	0.1474	0.5450
8	0.1399	0.5720
9	0.1333	0.5750
10	0.1272	0.5890

The table at the left shows the binding rate using probes from the Gator® BLI platform versus probes from the other BLI platform. At 1,000 ug/mL, Gator® probes show stable binding rates for 10 regenerations whereas the binding rates sequentially drop using the other BLI platform probes, thereby requiring a fresh probe.

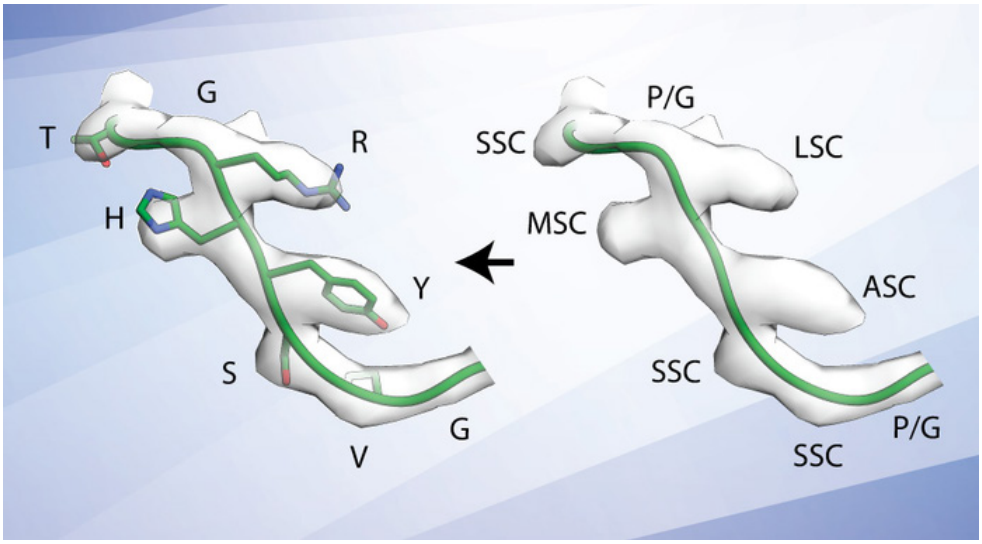
Hybrid Tech Uses Electron Microscopy and Next-Gen Sequencing to Speed Antibody Discovery



Release of monoclonal antibodies, illustration. [Nanoclustering/Science Photo Library/Getty Images]

Scientists at Scripps Research have devised a method that may be able to shortcut one of the big steps in modern vaccine development. The researchers showed that they could apply high-resolution, low-temperature electron microscopy (cryo-EM) data to rapidly characterize antibodies—elicited by a vaccine or infection—that bind to a desired target on a virus at an atomic level. The method utilizes a "structure-to-sequence" computer algorithm that can relate monoclonal antibody (mAb) structure determined by cryo-EM, to the DNA sequence that would produce that structure.

"The COVID-19 pandemic has highlighted the need for robust and rapid vaccine and antiviral technologies," said study senior author Andrew Ward, PhD, a professor in the Department of Integrative Structural and Computational Biology at Scripps Research. "We are optimistic that our new approach will help fill that need by greatly streamlining antibody discovery."



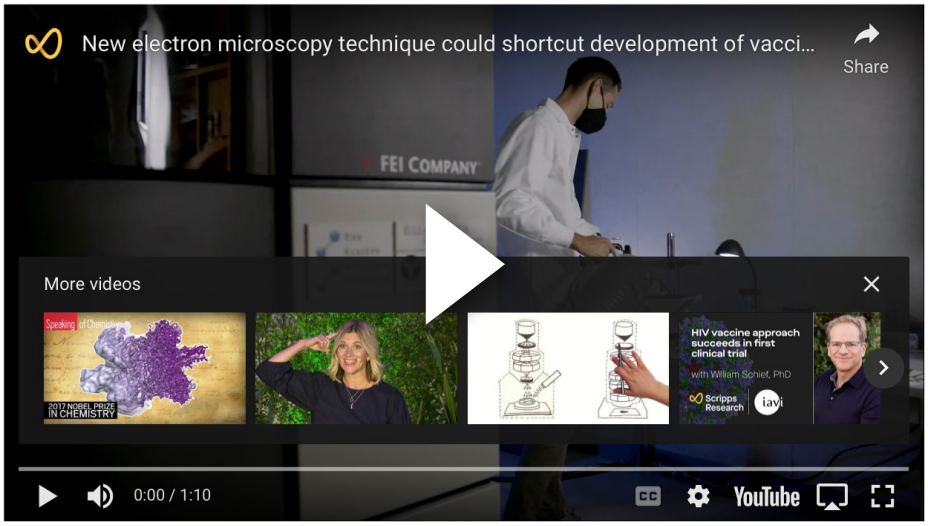
A new method developed at Scripps Research uses cryoEM technology to more quickly identify antibodies for use in vaccine development. In this example, the algorithm screened between approximately 100,000 to 1,000,000 possible antibody sequences from the database to identify the sequence (left) that best matches the antibody observed in their cryoEM images (transparent gray surface). [Scripps Research]

Ward and colleagues described their technology in Science Advances, in a paper titled "From structure to sequence: Antibody discovery using cryoEM."

A key rate-limiting steps in analyzing immune responses to vaccines or infections is the isolation and characterization of monoclonal antibodies, the authors noted. "One of the rate-limiting steps with traditional methods for antibody isolation is screening mAb libraries to identify the clones with desired epitope specificity … Comprehensive analyses of immune responses to infection or vaccination are laborious and expensive." As study co-first author, Aleksandar Antanasijevic, PhD, pointed out, "Traditionally, identifying antibodies that are useful against a virus involves the laborious sorting and testing of antibody-producing B cells to find the right ones—a process that takes months."

The researchers' feat was enabled in part by recent improvements in cryo-EM, a technology that uses a beam of electrons to illuminate and image targets far below the scale of ordinary light microscopy. In a study published in Nature Communications in August, for example, the researchers used high-resolution cryo-EM to rapidly and precisely map where antibodies in rhesus macaque monkeys bind to synthetic versions of the HIV envelope protein that are being developed for potential HIV vaccines. "Recently, we developed an approach that uses cryo–electron microscopy (cryoEM) for characterization of polyclonal antibody (pAb) responses elicited by vaccination or infection (cryoEMPEM) on the level of immune sera," they wrote.

For their new study, the investigators took this line of research a step further. They employed a "structure-to-sequence" computer algorithm that can relate antibody structure determined by cryo-EM, to the DNA sequence that would produce that structure. "In this study, we expanded the applicability of cryoEMPEM data by introducing a method for identification of functional antibody



The Scripps researchers showed that they could confirm the accuracy of the result by making copies of that unique, "monoclonal" antibody using the sequence data, and verifying with cryo-EM that the antibody binds in an identical way to the originally imaged antibody.

sequences from structural observations," they wrote.

To enable this, the team assembled a library of all the antibody-encoding DNA sequences from the rhesus macaque monkeys, which could obtained by quickly bulk sequencing the genetic material from antibody-producing B-cells from the animals' lymph nodes. Applying the algorithm to the cryo-EM data and the antibody sequence library, the scientists could reliably match an antibody of interest in their cryo-EM images to a unique antibody defined in the sequence database.

The researchers showed that they could confirm the accuracy of the result by making copies of a monoclonal antibody using the sequence data, and verifying with cryo-EM that the antibody bound in an identical way to the antibody that was originally imaged. " We developed a method to determine mAb sequences directly from cryoEMPEM maps," the team stated. "This hybrid approach, consisting of electron microscopy (EM) and next-generation sequencing (NGS), enabled sequence assignment of variable regions of polyclonal Fabs (Fv) including the complementarity-determining regions (CDRs) … This approach provides an alternative to traditional mAb discovery methods based on single B cell sorting, hybridoma, and phage display technologies."

"With this new method we can go from blood sample collection from infected or immunized patients to identifying all the elicited antibodies of interest in about ten days," stated staff scientist and study co-first author Charles Bowman, PhD.

The authors also noted that their reported proof of concept study used lymph node B cells with specificity for a particular antigen. However, they anticipate that their approach will work with B cells obtained from other sources, such as peripheral blood, spleen, bone marrow, or plasma cells, and without the presorting for

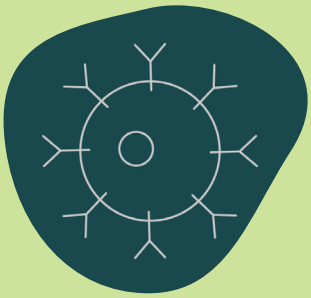
antigen binding. "By directly imaging the serum antibodies using cryoEM, we have a proxy for abundance, affinity, and clonality," they commented.

The scientists are now refining their technique to optimize its speed and usability, and are applying it to several areas: to rapidly evaluate human antibody responses to experimental HIV vaccines; to develop antibody-blocking treatments for autoimmune diseases; and to discover antibodies that could therapeutically hit other protein targets on cells.

They expect that future improvements in cryo-EM technology and structure-to-sequence algorithms will allow the even more rapid identification of antibodies using high-resolution structural images alone, with no need for DNA sequencing of B cells.

"This structure-to-sequence approach has a lot of potential in immunology and beyond," Antanasijevic said. "We envision being able to use it someday to study protein-to-protein interactions generally, for example, to discover a given protein's binding partners." As the authors concluded, " … our approach will open up new doors for both the discovery of mAbs and analyzing antibody responses to infection and vaccination. The ongoing COVID-19 pandemic has highlighted the need for such robust and rapid technologies."

A key rate-limiting steps in analyzing immune responses to vaccines or infections is the isolation and characterization of monoclonal antibodies, the authors noted. "One of the rate-limiting steps with traditional methods for antibody isolation is screening mAb libraries to identify the clones with desired epitope specificity… Comprehensive analyses of immune responses to infection or vaccination are laborious and expensive.



Gator® Flex SA Kit: A Reactivable Streptavidin Probe Kit

INTRODUCTION

Many of the label-free probes have been successfully regenerated to reduce the cost of drug discovery and development. However, extensive application of streptavidin (SA) probes is still hindered by the irreversible immobilization of the ligand molecule, and the ability to reuse the probe for different applications is desirable for cost-effective research.

GATOR® FLEX SA KIT

The Gator® Flex SA Kit is the first on the market to provide reactivable streptavidin probes with a set of reagents. One probe can easily be reused for different applications, which makes it beneficial for academics and for utility in fully automated screening of different biomolecular interactions.

PERFORMANCE SUMMARY

- Removes both capture reagent and biotinylated protein, and immobilizes new capture reagent in under 5 minutes
- Uses the same or different biotinylated protein
- Can be reused at least 10 times without the loss of performance
- Automated reactivation of the probe
- Reagents can be stored at 4°C for up to 5 months

Results

LOADING HEIGHT REPRODUCIBILITY

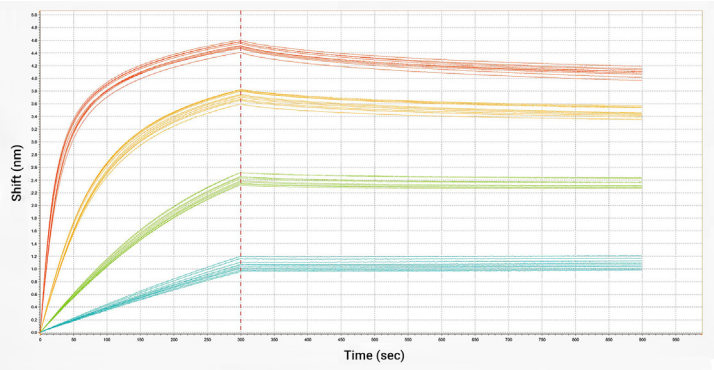
Three Flex SA probes were reactivated over 10 times using biotinylated TNF-α at 1 µg/mL. A loading height of coefficient of variation (CV) <10% suggests excellent reproducibility.

	Probe 1 (n=10)	Probe 2 (n=10)	Probe 3 (n=10)
Loading Height Avg (nm)	0.66	0.67	0.62
Loading Height SD (nm)	0.03	0.03	0.03
Loading Height %CV	4.54	4.09	5.40

Loading height precision using 3 independent Flex SA probes.

REPRODUCIBILITY OF K_D OVER 10 REACTIVATIONS USING THE SAME BIOTINYLATED LIGAND

Flex SA Capture reagent was loaded onto the Flex SA probe, followed by biotinylated TNF-α. K_D values for TNF-α binding with anti-TNF-α IgG at 10, 30, 100 and 300 nM concentrations were obtained. Global fit analysis using the GatorOne software resulted in K_D = 7.63E-10 M for over 10 reactivations.



Kinetics characterization of biotinylated TNF-α and anti-TNF-α for over 10 reactivations.

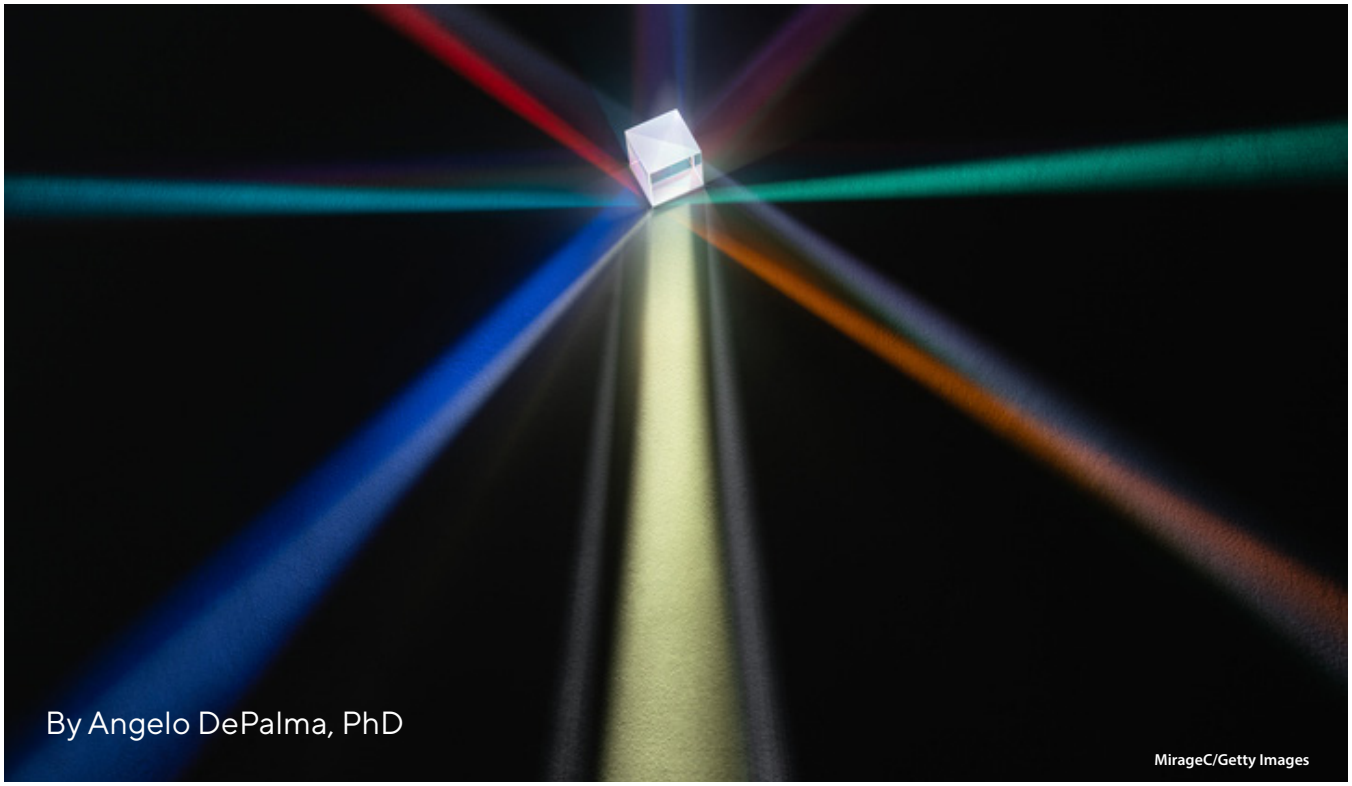
K_D MEASUREMENTS FOR DIFFERENT PROTEINS OVER 10 REACTIVATIONS

The same probe was tested with 2 different proteins for over 10 reactivations.

Biotinylated PDL1 : Anti-PDL1		Biotinylated CRP : Anti-CRP	
Reactivation 1	2.00E-10	Reactivation 2	4.77E-10
Reactivation 3	1.08E-10	Reactivation 4	4.28E-10
Reactivation 5	1.17E-10	Reactivation 6	3.88E-10
Reactivation 7	1.06E-10	Reactivation 8	3.49E-10
Reactivation 9	0.40E-10	Reactivation 10	3.22E-10
Average	1.14E-10	Average	3.93E-10

K_D measurements using the same set of Flex SA probes with 2 different kinetics pairs. The 2 pairs were used alternately for over 10 reactivations.

Protein Titer Monitoring through Index of Refraction



By Angelo DePalma, PhD

MirageC/Getty Images

Among the various sensing modalities available for bioprocess operations, index of refraction (IoR) is intriguing for its simplicity and capability for sample-less, real-time readout. A group led by Steven Harris of Pall showed in a recent publication that continuous, in-line IoR monoclonal antibody concentration measurement was feasible and, if combined with adaptive control, "could be used to ensure a consistent purification process performance with a seamless advanced process control."

GEN asked Harris, lead author and principal scientist, why bioprocessors may have overlooked IoR—a legacy analytical technology if there ever was one—until now.

"Refractometry is not commonly used in determining protein concentration in biomanufacturing because the signal is not specific for proteins (such as monoclonal antibodies), meaning that matrix components and host cell proteins all contribute to the signal," he says. "We demonstrated that this limitation may be mitigated by measuring the refractive index in chemically defined media, for example, the chromatography elution buffer, and by using refractometry after the Protein A affinity capture

step, at which point nearly all of the other host cell proteins have been removed."

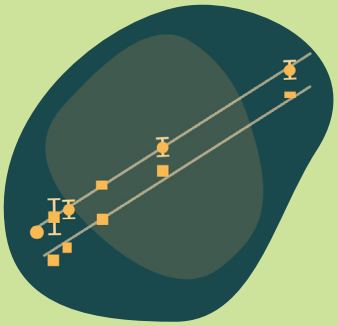
Process analytic technology and quality by design were promulgated in the first decade of the 2000s. Why has adoption been so slow?

According to Harris, as with most innovations in biomanufacturing, developers prefer being a close second to being first. "The upfront investment in money, time, and manpower to adopt PAT and QbD is quite high.

Although companies recognize their potential benefits, the risks involved in being first is not negligible. This risk is balanced by not moving too fast or too far ahead of the industry standard. This, along with validation requirements, results in a slower pace of adoption."

The case for continuous measurement rests on the presumption that upstream protein output will change over the course of the production run. Harris notes that while textbook perfusion processes generate consistent titers, "even the most well-defined and controlled bioreactor is still a variable process of living organisms. The goal of control platforms is to minimize the impact of that variability and, especially in the perfusion paradigm, to keep the cells as happy as possible for a long as possible. Ideally the upstream titer output will be maintained within the downstream operating titer range."

Among the various sensing modalities available for bioprocess operations, index of refraction (IoR) is intriguing for its simplicity and capability for sample-less, real-time readout. A group led by Steven Harris of Pall showed in a recent publication that continuous, in-line IoR monoclonal antibody concentration measurement was feasible and, if combined with adaptive control, "could be used to ensure a consistent purification process performance with a seamless advanced process control."



Learn more about how Gator BLI technology can measure concentrations of purified proteins in real time here

Gator® Next-Gen HFC Probes

INTRODUCTION

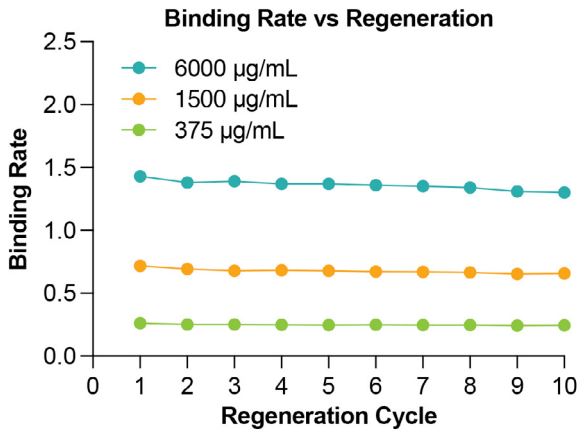
Gator® Anti-Human IgG FC Gen II (HFCII) Probes are high-performance, nanobody-based biosensors that can detect and quantitate human IgG isotypes in different buffers (Q Buffer and K Buffer), cell culture supernatant, cell media, and other crude samples.

The enhanced kinetics sensitivity, higher dynamic range for quantification, and better regeneration capabilities make these probes highly desirable in high-throughput applications such as epitope mapping/binning, lead-to-hit discovery and optimization, and cell line development.

PERFORMANCE SUMMARY

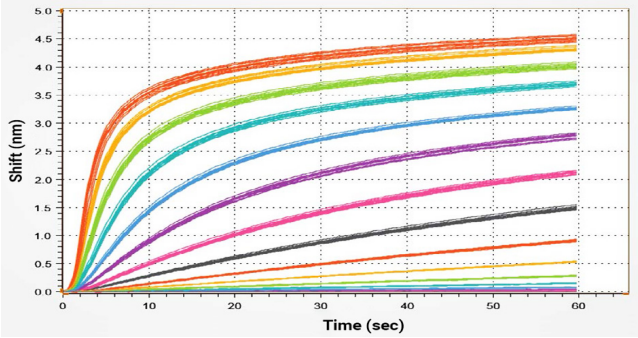
- Dynamic range: 0.3 - 6000 µg/mL in Q buffer; 1 - 2000 µg/mL in diluted cell culture media
- Limit of detection (LOD): 0.1 µg/mL
- Crude sample tolerant
- Time to Result: 8 samples in 4 minutes, 96 samples in 26 minutes
- Cost-effective: Reusable at least 20 times in Q, K Buffer, and diluted cell culture media

Regeneration Performance



Binding rate of human IgG (6000, 1500, and 375 µg/mL) to HFCII probes. No loss of binding was observed after 10 regeneration cycles.

Quantification

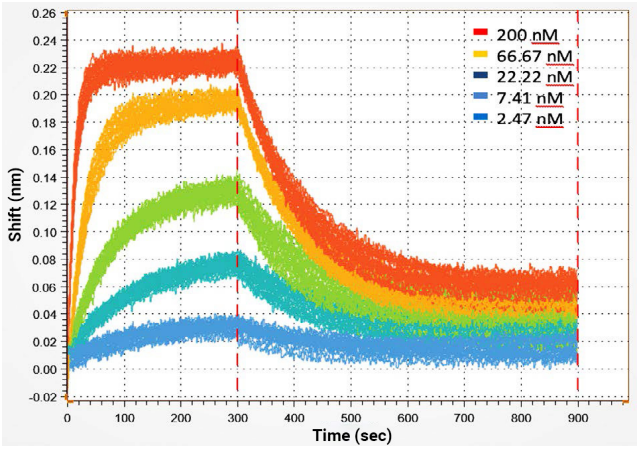


Quantitation of human IgG in Q Buffer. IgG concentrations range between 0.3 and 6000 µg/mL. The data was acquired in 60 seconds at 400 rpm.

Known Conc (mg/mL)	Avg Binding Rate	%CV	Calc Conc (mg/mL)	%CV
6000.00	1.3600	2.80	6815.00	8.30
3000.00	1.0000	2.40	3252.00	5.10
1500.00	0.6767	2.70	1575.00	4.60
750.00	0.4233	2.90	779.60	4.00
375.00	0.2485	2.10	389.80	2.60
188.00	0.1398	2.60	197.50	2.90
93.80	0.0722	2.30	94.70	2.50
46.90	0.0383	2.40	48.10	2.60
23.40	0.0199	1.50	24.40	1.50
11.70	0.0103	0.80	12.40	1.00
5.86	0.0051	2.00	6.10	2.00
2.93	0.0025	1.50	2.97	1.50
1.46	0.0012	2.00	1.50	1.80
0.73	0.0006	3.10	0.84	2.90
0.37	0.0003	8.20	0.44	7.10

Percent coefficient of variation (% CV) of human IgG binding to HFCII and calculated concentrations are <10 % over 20 regenerations.

Kinetics

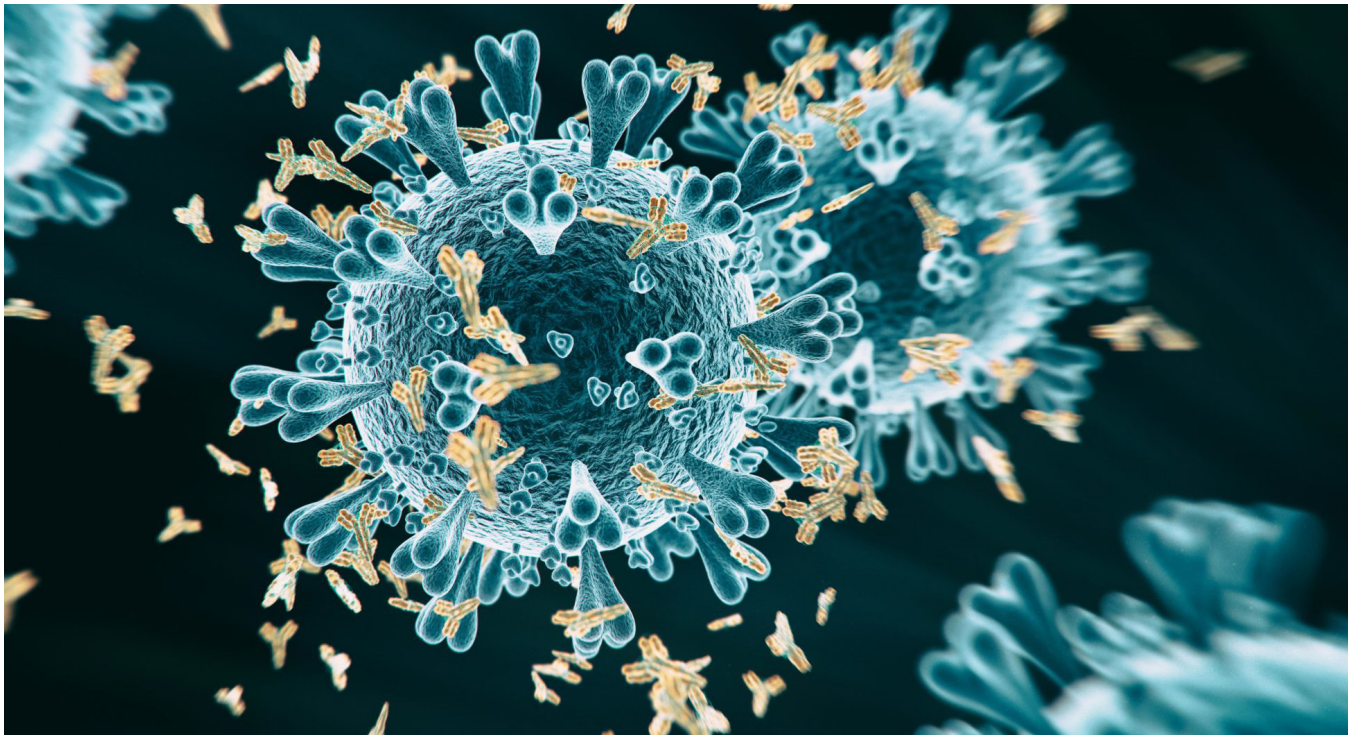


Association and dissociation of anti-RBD IgG1 (5 µg/mL) and RBD protein (2.47 - 200 nM; 1:3 dilution) measured at 1000 rpm for over 20 regeneration cycles.

Cycles	k_{off} (1/s)	k_{on} (1/Ms)	K_D (M)
1	2.82E-03	3.70E+05	7.62E-09
2	2.84E-03	3.78E+05	7.50E-09
3	2.97E-03	3.87E+05	7.68E-09
4	2.65E-03	4.08E+05	6.49E-09
5	2.66E-03	4.08E+05	6.53E-09
6	3.20E-03	4.30E+05	7.44E-09
7	3.14E-03	4.05E+05	7.75E-09
8	3.31E-03	4.48E+05	7.38E-09
9	3.19E-03	4.39E+05	7.27E-09
10	2.85E-03	5.01E+05	5.69E-09
11	4.23E-03	4.26E+05	9.93E-09
12	3.92E-03	4.23E+05	9.26E-09
13	3.73E-03	4.46E+05	8.37E-09
14	3.34E-03	5.01E+05	6.67E-09
15	3.76E-03	4.62E+05	8.14E-09
16	4.33E-03	4.41E+05	9.80E-09
17	4.46E-03	4.46E+05	9.99E-09
18	3.71E-03	5.08E+05	7.29E-09
19	3.50E-03	5.43E+05	6.44E-09
20	3.91E-03	4.97E+05	7.86E-09

Kinetics parameters for anti-RBD IgG1 and RBD protein for over 20 regeneration cycles. k_{off}, k_{on} and K_D values are within 10- fold of each other.

Cross-Reactive Antibody Blocks Infections against Multiple Coronaviruses in Mice



Release of monoclonal antibodies, illustration. [Nanoclustering/Science Photo Library/Getty Images]

Given the more than 200 million people that have been infected with SARS-CoV-2 globally, resulting in over 4.5 million deaths, the development of effective treatments for the infection is a global health priority. In some cases, monoclonal antibody treatments have been an effective treatment for COVID-19. But the emergence of SARS-CoV-2 variants that are partially or fully resistant to some neutralizing antibodies authorized for COVID-19 treatment bring to the forefront the need to develop cross-reactive monoclonal antibodies that are broadly effective against existing SARS-CoV-2 variants and zoonotic SARS-related viruses that may emerge in the future.

Now, a research collaboration between scientists at the Duke Human Vaccine Institute (DHVI) at Duke University and the University of North Carolina (UNC) at Chapel Hill has identified and tested an antibody that limits the severity of infections from a variety of coronaviruses, including those that cause COVID-19 as well as the original SARS illness.

This work is published in Science Translational Medicine in the article, "A broadly cross-reactive

antibody neutralizes and protects against sarbecovirus challenge in mice."

"This antibody has the potential to be a therapeutic for the current epidemic," said co-senior author Barton Haynes, MD, director of DHVI. "It could also be available for future outbreaks, if or when other coronaviruses jump from their natural animal hosts to humans."

The antibody was isolated by analyzing the blood from a patient who had been infected with the original SARS-CoV-1 virus, which caused the SARS outbreak in the early 2000s, and from a current COVID-19 patient. The team identified more than 1,700 monoclonal antibodies. Of the 1,700 antibodies from the two individuals, the researchers found 50 antibodies that had the ability to bind to both the original SARS virus and SARS-CoV-2.

David Martinez, PhD, a post-doctoral researcher in the Baric lab at UNC Gillings School of Global Public Health stated, on Twitter, that they found, "several antibodies that cross-reacted with SARS-CoV, SARS-CoV, bat CoV RsSHC014, RaTG13, and Pangolin spike proteins."

Further analysis found that one of those cross-binding antibodies was especially potent and able to bind to a multitude of animal coronaviruses in addition to the two human-infecting pathogens. "This antibody binds to the coronavirus at a location that is conserved across numerous mutations and variations," Haynes said. "As a result, it can neutralize a wide range of coronaviruses."

Martinez tweeted that one antibody, named DH1047, neutralized alpha (B.1.1.7), beta (B.1.351), gamma (P.1), epsilon (B.1.429), iota (B.1.526), kappa (B.1.617.1), and the highly transmissible delta variant (B.1.617.2). The structure of DH1047 was solved when bound to SARS-CoV by CryoEM. He noted that the epitope bound by DH1047 is an Achilles Heel of sarbecoviruses and could be a template for a universal vaccine.

With the antibody isolated, the DHVI team turned to researchers at UNC who have expertise in animal coronaviruses. The UNC team, led by coronavirus expert Ralph Baric, PhD, epidemiology professor at UNC Gillings School of Global Public Health, tested it in mice to determine whether it could effectively block infections, or minimize the infections that occurred.

They found that it did both. When given before the animals were infected, the antibody protected mice against developing SARS, COVID-19, and its variants such as Delta, and many animal coronaviruses that have the potential to cause human pandemics.

"The findings provide a template for the rational design of universal vaccine strategies that are variant-proof and provide broad protection from known and emerging coronaviruses," Baric said.

When given after infections, the antibody reduced severe lung symptoms compared to animals that were not treated with the antibody.

"The therapeutic activity even after mice were infected suggests that this could be a treatment deployed in the current pandemic, but also stockpiled to prevent the spread of a future outbreak or epidemic with a SARS-related virus," said Martinez. "This antibody could be harnessed to prevent maybe SARS-CoV-3 or SARS-CoV-4."

Gator® Ni-NTA Kit for Quantitation and Kinetics Analysis of His-tagged Proteins

INTRODUCTION

Polyhistidine tag (His-Tag) on the N- or C-terminus of proteins is commonly used for immobilized metal ion affinity chromatography (IMAC).
The Gator® Ni-NTA probes allow for rapid and continuous quantification of His-tagged proteins without the need for nickle (Ni^{2+}) recharging step by using Gator® Ni-NTA Regen Buffer and Ni-NTA Neutral Buffer. The stable immobilization of His-tagged proteins allows for facile kinetics analyses with binding partners, off-rate screening, and epitope binning of antibodies experiments.

GATOR® Ni-NTA PROBES

Gator® Ni-NTA Probes are functionalized with Qiagen™ Tris-NTA and charged with Ni^{2+} ions for high affinity immobilization of His-tagged proteins. The probes can be used for quantitation of His-tagged proteins in sample buffer and cell culture supernatants. The specially formulated Gator® Ni-NTA Regen and Ni-NTA Neutral Buffers allow for continuous regeneration of the probes, without the need for an additional Ni^{2+} recharging step.

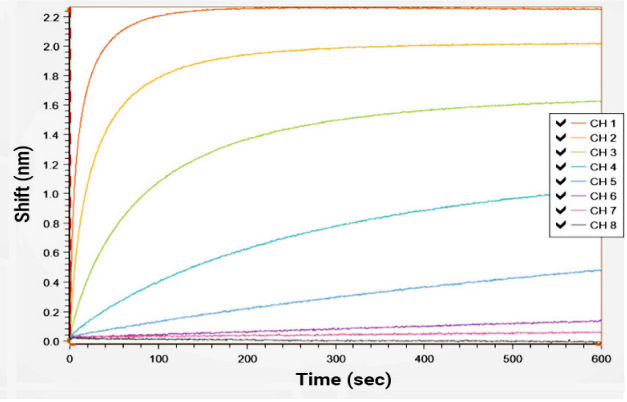
PERFORMANCE SUMMARY

- Dynamic range: 0.25 - 1000 µg/mL (in Q buffer); 1 - 1000 µg/mL (in diluted cell culture media)
- Time to Result: 8 samples in 4 minutes, 96 samples in 26 minutes
- Limit of detection (LOD): 0.25 µg/mL (10 minutes, 1500 rpm)
- Crude sample tolerant
- Cost-effective: reusable at least 20 times by regeneration in Q buffer and 10 times in diluted cell culture media

RESULTS

DYNAMIC RANGE

The dynamic range of Ni-NTA probes was tested using His-tagged Protein A diluted in Q Buffer. The data was acquired in 10 minutes at 1500 rpm.



Detection of His-tagged Protein A standards using Ni-NTA probes.

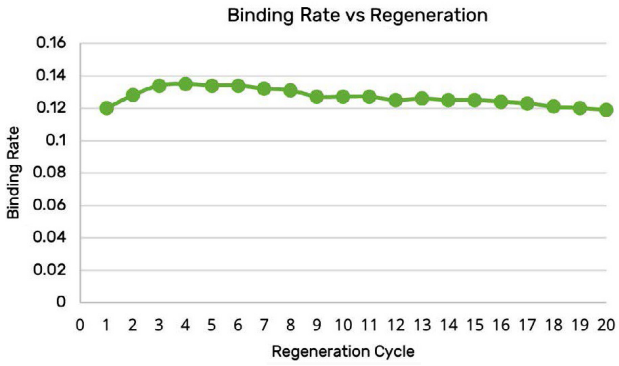
Known Conc (mg/mL)	Calculated Conc (mg/mL)	Binding Rate
1000.00	1018.31	0.3109
250.00	250.37	0.1038
62.50	65.73	0.0266
15.63	15.46	0.0051
3.91	4.05	0.0011
0.98	1.08	0.0003
0.24	0.25	0.0001

Calculated concentrations and binding rates for concentrations 0.24 µg/mL - 1000 ug/mL.

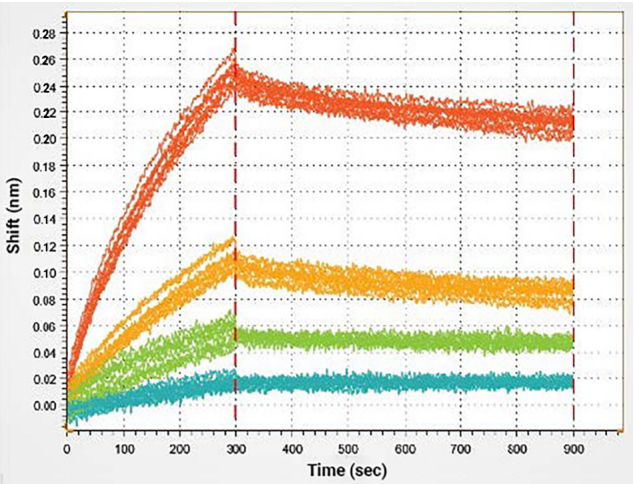
REGENERATION PERFORMANCE

Quantitation

Quantitation performance after 20 regenerations from the detection of the same concentration of His-tagged Protein A using Ni-NTA probes.
No loss in binding rate is observed even after 20 regenerations.



Binding rate results through 20 regeneration cycles from the detection of His-tagged Protein A using Ni-NTA probes.



Kinetics interaction between His-tagged CD64 with human IgG after 10 regenerations.

Kinetics

Similar kinetics parameters (k_{on}, k_{off}, K_D) of the binding between His-tagged CD64 with human IgG were obtained following 10 regeneration cycles.

Cycles	k_{off} (1/s)	k_{on} (1/Ms)	K_D (M)
1	2.54E-04	1.39E+04	1.83E-08
2	3.34E-04	2.25E+04	1.48E-08
3	3.25E-04	1.39E+04	2.34E-08
4	2.78E-04	2.00E+04	1.39E-08
5	3.05E-04	1.78E+04	1.71E-08
6	2.72E-04	1.66E+04	1.64E-08
7	2.58E-04	1.59E+04	1.62E-08
8	2.68E-04	1.48E+04	1.81E-08
9	2.90E-04	1.78E+04	1.63E-08
10	3.04E-04	2.17E+04	1.40E-08

Kinetics results for the interaction between His-tagged CD64 with human IgG using Ni-NTA probes after 10 regenerations.

Gator Bio 21 CFR Part11 Compliant Total Solution

*Gator® Pro CFR Part11 coming soon

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