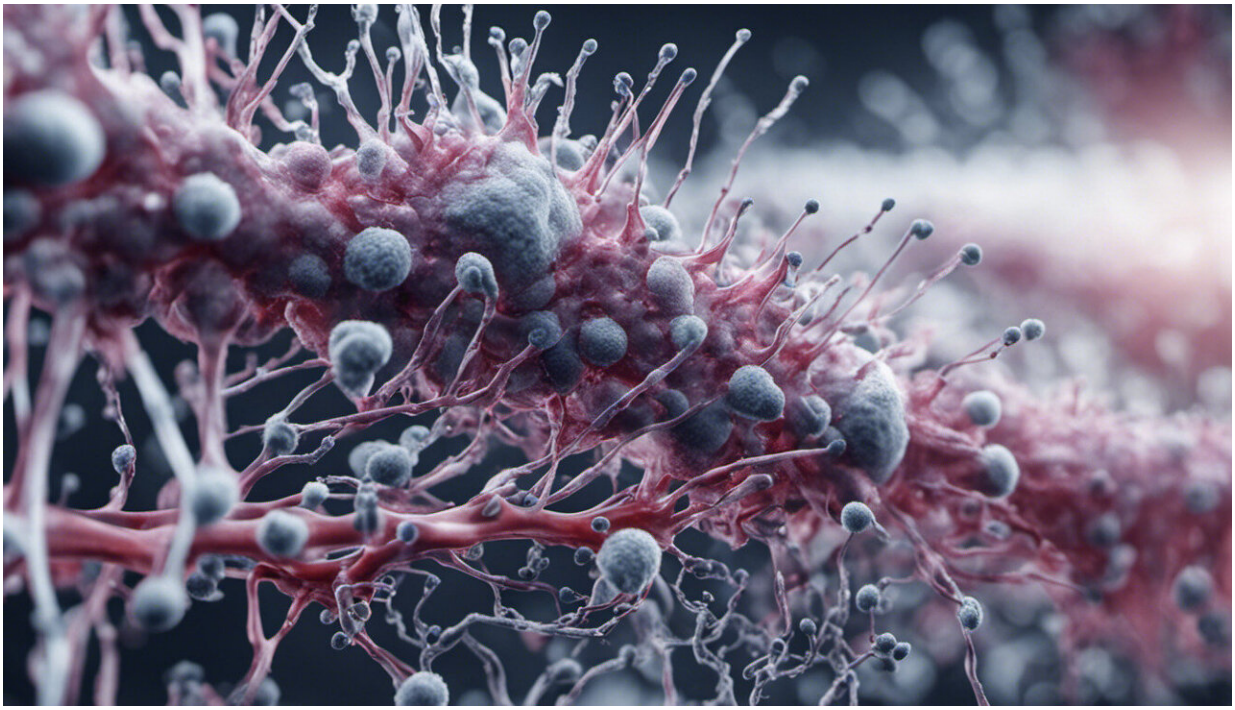
Researchers use X-rays to find the best antibodies

July 26 2023, by Kevin Jackson



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Antibody therapies have a variety of uses, but we need to know which therapies work and which ones don't. Recent research has discovered a means to determine how effective certain antibodies can be in medical scenarios.

Imagine you're going to a party where you only know a single person. While you've been friends with this person for a long time, you're concerned about them leaving you alone in a room full of strangers. Even though they promise they'll stick by your side, they immediately walk away from you upon entering the party to speak to someone else they know.

In such a situation, wouldn't it be nice to have a scientific guarantee that you won't be left to sulk in the corner alone?

In a certain sense, this scenario mirrors the problem that scientists have with antibody research. They want these antibodies to perform a specific job, but they can sometimes drift off like your friend and bind with the wrong molecules.

For an overactive antibody, this is called polyreactivity—and it's a topic of interest for Andrew Kruse, a professor of Biological Chemistry and Molecular Pharmacology at Harvard Medical School.

"Polyreactivity is the phenomenon where an antibody binds to a broad range of molecules that aren't its intended target," Kruse said. "This is a common phenomenon for many antibodies. It's a major liability in drug development, and we would prefer to work with antibodies that are not polyreactive."

Kruse and his colleagues are interested in avoiding polyreactivity because doing so can save other researcher's time. Antibody therapies are important in treating sick people suffering from diseases such as COVID-19. But a lot of in-person laboratory work must be performed to decide if a particular antibody treatment is effective, and this takes a lot of time. Studies such as those led by Kruse allow scientists to pick the antibodies that are most likely to be effective, and therefore not waste time on polyreactive antibodies.

For this research, which has been published in *Nature Communications*, the team made use of the Advanced Photon Source (APS), a U.S. Department of Energy (DOE) Office of Science user facility at DOE's Argonne National Laboratory.

Finding the right fit

In their research, Kruse and his colleagues wanted to identify the antibodies that were highly polyreactive. This would allow them to discard these antibodies as potential treatment tools.

To begin, the researchers used a method called yeast display to isolate certain antibody fragments for further study. The scientists were able to sort the antibody fragments based on their level of polyreactivity, which thereby enabled the training of a machine learning model to predict which features in antibody sequences contribute to polyreactivity.

For our metaphor, you can imagine this as if you had a friend who had demonstrated at multiple previous parties that they would never leave your side.

What's more, the researchers weren't just looking at each individual antibody—they were also looking at mutations of each.

"One antibody that we'd been working with in my lab is an antibody called AT118," Kruse said. "This is a single domain antibody fragment that binds to the angiotensin II type one receptor. It's basically a research tool that we've used for looking at how modulating that receptor can affect blood pressure. It has anti-hypertensive effects that we were interested in, and we had seen previously that it was a bit sticky."

Kruse continues, "It binds to a variety of different things, and so we thought we could take that sequence, run it through our models, and try

to predict mutations that would decrease its reactivity. We did that, and we came up with several mutations that lowered polyreactivity while preserving the antibody's ability to bind to its target. Then, we wanted to understand on a molecular mechanistic level how those mutations work. Why does this certain substitution change the reactivity properties of this antibody? To do that, we crystallized the antibody, we solved the structure at the APS, and we were able to see how these mutations actually changed the reactivity properties of this clone."

In this way, the researchers were able to find certain mutations for specific antibodies that were less likely to exhibit polyreactivity.

Craig Ogata, a protein crystallographer at Argonne, says that the protein crystallography used at the APS is a well-established means of determining the structure of large molecules. These structural details of antibodies and how they bind to specific sites can tell scientists more about the contact points on both the antibody and the foreign molecule that it's binding to. Therefore, knowing more about these contact points can help scientists determine how to block or reduce the interactions of a foreign protein.

Crystals of these antibody molecules were analyzed at the APS, where the research team used the bright X-ray beams of the National Institute of General Medical Sciences and National Cancer Institute Structural Biology Facility (GM/CA) to collect diffraction data. This diffraction data was processed by powerful computers to give the scientists a structural understanding of the molecules, which can help with understanding the polyreactivity of a given antibody.

What comes next?

While being able to study the polyreactivity of these antibodies will enable scientists to shave time off their research, this work has

implications far beyond that. For instance, Kruse discusses the possibility of using this technique to identify antibodies that are ripe for modification.

"One application that we had in mind is if you have an antibody that has interesting biological properties, but has some level of polyreactivity that you'd like to engineer out," Kruse said. "We could use these models, then, to predict mutations that might rescue an otherwise problematic antibody."

More information: Edward P. Harvey et al, An in silico method to assess antibody fragment polyreactivity, *Nature Communications* (2022). DOI: 10.1038/s41467-022-35276-4

Provided by Argonne National Laboratory