No llamas required—Researchers develop alternate method to uncover protein structures, design new drugs

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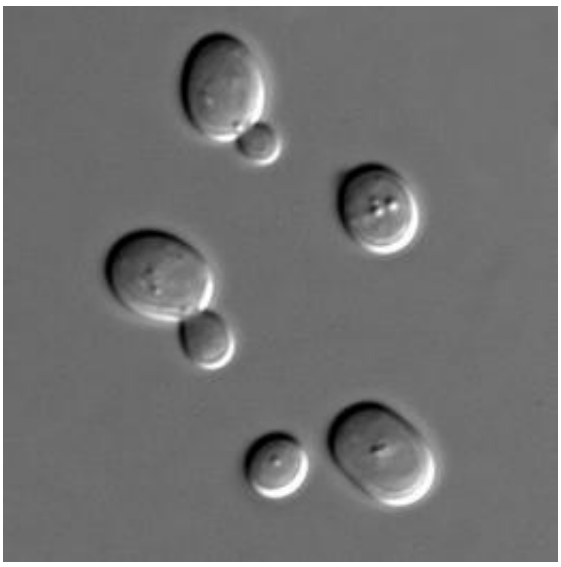

Sacharomyces cerevisiae cells in DIC microscopy. Credit: Wikipedia.

Detouring around a major research roadblock, researchers have found a new way to create valuable antibodies without needing ... llamas?

It is a little-known fact that llamas, alpacas, camels and other members of the camelid family make a unique class of antibodies that allow scientists to determine the structures of otherwise impossible-to-study proteins in the body, understand how those proteins malfunction in disease and design new drugs that act on them.

As one might imagine, there are downsides to taking advantage of this evolutionary happenstance.

First, not all researchers who need camelid antibodies for their experiments have access to llama (or alpaca or camel) facilities. Second, while the animals aren't harmed, vaccinating them to generate the desired antibodies is expensive, takes as long as six months per attempt and often doesn't work.

So, biochemists Andrew Kruse at Harvard Medical School and Aashish Manglik at the University of California, San Francisco, teamed up to create a llama-free solution: vials of specially engineered yeast.

The yeast method, described Feb. 12 in *Nature Structural and Molecular Biology*, can be done in a test tube in a researcher's own lab. It has a higher success rate and faster turnaround time than both llama vaccination and previous attempts to circumvent camelids, the authors say.

It also marks the first time a camelid-bypass system has been made freely available for nonprofit use.

"There's a real need for something like this," said Kruse. "It's low-tech, it's a low time investment and it has a high likelihood of success for most proteins."

"People who have struggled to nail down their protein structures for years with llamas are getting them now," he said.

Lock and key

The active segments of camelid antibodies are often called nanobodies because they can be much smaller than regular antibodies. A llama

nanobody might bind only to a particular conformation—for example, "open" or "closed"—of a particular protein. Nanobodies also can bind to challenging proteins, such as receptors that work in oily cell membranes.

Structural biologists like Kruse and Manglik want to find the exact nanobody that matches their protein of interest so they can lock the protein in one position and run tests to figure out its atomic structure. Learning the structure allows them to study how the protein works and provides a blueprint for designing drugs that target it.

Nanobodies have opened long-locked doors in biomedical science. For example, they have allowed researchers to see for the first time how neurotransmitters such as adrenaline and opioids bind to receptors in the brain.

Scientists just needed an easier way to find the keys.

Glowing success

Right now, a scientist who wants to study a difficult membrane protein has to laboriously generate several milligrams of it, inoculate a llama with it—usually done through a third-party service—and hope the animal's immune system responds. Only then can she search for antibodies in a blood sample and hope there are enough to work with.

By contrast, Kruse and Manglik's research team, led by first author Conor McMahon, a postdoctoral researcher in the Kruse lab, created a library of 500 million camelid antibodies using yeast cells.

Each yeast cell has a slightly different nanobody tethered to its surface, made by a slightly different piece of synthetic DNA.

The researchers mixed all the yeasts together and froze them for

safekeeping. Anytime they want to run an experiment, they simply defrost a test tube's worth: a miniature llama immune system. (The tube contains 10 to 20 times the amount needed to ensure that at least one of each unique antibody is included.)

The team developed a method where, instead of injecting a llama, scientists can now label their protein of interest with a fluorescent molecule and add it to the test tube. Yeast with surface nanobodies that recognize the protein will glow.

The researchers then use fluorescence-activated cell sorting, or FACS, to separate the glowing yeast from the rest.

They sequence the DNA of those glowing yeast cells to learn what the nanobodies are. They can then use E. coli bacteria to grow as many of those nanobodies as they need.

The whole process takes three to six weeks instead of three to six months.

Money for nothing, and yeast for free

The team tested its yeast system on two proteins: the beta-2 adrenergic receptor, linked to asthma, and the adenosine receptor, which is a gateway for caffeine to deliver its buzz. In both cases, the nanobody bound to the desired receptor, bound only to that receptor, and bound to it only when it was "on."

"We found that the yeast-derived nanobodies can do everything llama -derived antibodies can," said Kruse.

The team is now offering vials of the yeast mix and usage instructions free of charge to any nonprofit labs that want them. Commercial

companies can license the yeast. "We made a big batch," said McMahon, so there's plenty to go around.

More than 40 labs requested vials before the paper was even published.

"Nanobodies are making it possible to develop drugs for biological targets that antibodies were simply too big to hit," said Manglik. "By making nanobody discovery quick and easy, we hope our platform will dramatically accelerate the potential applications of this exciting technology."

"I think we'll see things that blow the animal immune system away," added McMahon. "This is new technology. It's only going to get better. Hopefully it will work as well or better so we won't need llamas anymore."

More information: Yeast surface display platform for rapid discovery of conformationally selective nanobodies, *Nature Structural and Molecular Biology*, nature.com/articles/doi:10.1038/s41594-018-0028-6

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