An easier way of sneaking antibodies into cells

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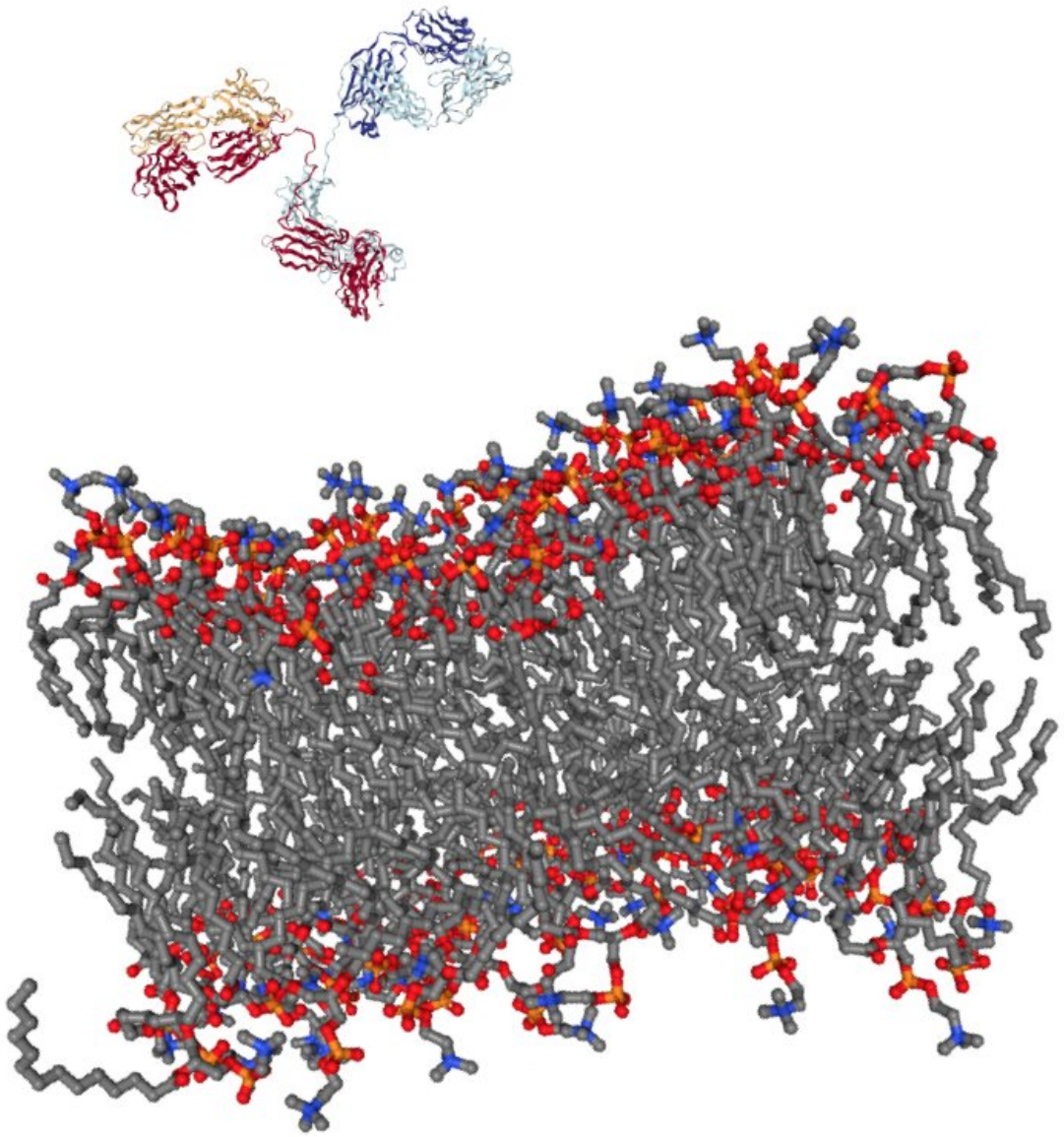

Getting a complex protein like an antibody through the membrane of a cell without damaging either is a long-standing challenge in the life sciences. Penn Engineers have found a plug-and-play solution that makes antibodies compatible with the delivery vehicles commonly used to ferry nucleic acids across that barrier. Credit: University of Pennsylvania

For almost any conceivable protein, corresponding antibodies can be developed to block it from binding or changing shape, which ultimately prevents it from carrying out its normal function. As such, scientists have looked to antibodies as a way of shutting down proteins inside cells for decades, but there is still no consistent way to get them past the cell membrane in meaningful numbers.

Now, Penn Engineering researchers have figured out a way for antibodies to hitch a ride with transfection agents, positively charged bubbles of fat that biologists routinely use to transport DNA and RNA into cells. These delivery vehicles only accept cargo with a highly negative charge, a quality that nucleic acids have but antibodies lack. By designing a negatively charged amino acid chain that can be attached to any antibody without disrupting its function, they have made antibodies broadly compatible with common transfection agents.

Beyond the technique's usefulness towards studying intracellular dynamics, the researchers conducted functional experiments with antibodies that highlight the technique's potential for therapeutic applications. One antibody blocked a protein that decreases the efficacy of certain drugs by prematurely ejecting them from cells. Another blocked a protein involved in the transcription process, which could be an even more fundamental way of knocking out proteins with unwanted effects.

The study, published in the *Proceedings of the National Academy of Sciences*, was conducted by Andrew Tsourkas, professor in the Department of Bioengineering, and Hejia Henry Wang, a graduate student in his lab.

"If you want to study the role of a specific protein within a cell, you need a way of shutting down its function," Tsourkas says. "Scientists have been talking about using antibodies as a way of doing that since at least the 1980s, but even the best methods today are inefficient and often invasive."

These most effective techniques to date involve manually injecting antibodies into a cell's cytoplasm with a microscopic needle, a painstaking effort that is impossible to do at large scales. Pulses of high-voltage electricity can also be used to temporarily form holes in plasma membranes to allow antibodies to flow into the cytoplasm, but requires significant tinkering to ensure the cells aren't killed in the process.

Tsourkas and Wang were inspired by the possibilities of intracellular antibody delivery, but looked towards the success of commercial transfection agents that have been developed for gene delivery, which has allowed countless studies of cellular dynamics by introducing new sequences of DNA or RNA into cells.

"Exploiting the technical developments achieved in gene delivery would allow us to deliver antibodies to a much larger population of cells," Tsourkas says. "More important, being able to ferry antibodies across the cellular membrane as easily as DNA or RNA would open up a world of experiments that is currently just out of reach of most biology labs."

Entirely blocking the production of proteins using DNA and RNA is a blunt instrument. Antibodies, however, can be used to inhibit specific protein functions or block only relevant binding sites.

"Because antibodies are so widely used in biological research, there are tens if not hundreds of thousands of well-validated ones available off the shelf from commercial vendors," Wang says. "Our technique allows researchers to use any of these antibodies to inhibit intracellular proteins for the first time, greatly expanding the uses for existing antibody collections."

At the core of the Penn Engineers' technique was finding a plug-and-play solution to the main barrier that prevents antibodies and other proteins from being compatible with commercial transfection agents. Those positively charged lipid bubbles readily accept DNA and RNA because nucleic acids have a strong negative charge, whereas antibodies have a range of positive and negative charges throughout their structures.

A small amount of previous research from other labs had shown the potential of attaching a highly negative "label" to proteins, changing their overall charge enough to work with commercially available transfection agents. Tsourkas and Wang extended this research by designing a simple way to attach anionic polypeptides—short chains of negatively charged amino acids—to nearly any existing antibody.

"If you're not an antibody production lab, you probably don't have the necessary expertise to confidently create an antibody with a negatively charged label," Tsourkas says. "What we've done is come up with a very simple reagent to precisely attach anionic polypeptides to any of the antibodies you might buy off the shelf in a single step, without blocking its function in the process."

The earlier, painstaking techniques for getting antibodies into cells had success rates in the single digits. By contrast, the Penn Engineers' experiments saw up to 90 percent of the cells end up with enough antibodies to inhibit the protein function that was being targeted.

"In one case, we inhibited a protein that clears small-molecule drugs before they can take effect. Drug-response curves showed that we could kill the cells with a five-times lower dose than if that protein was active," Tsourkas says. "In our second case, we showed that we could prevent a transcription factor from getting into the cell's nucleus, where it would normally transcribe RNA, by measuring the amount of the protein that the RNA codes for."

The team has recently been awarded a grant to extend this work to in vivo studies in mice. There, they will have to develop new delivery vehicles that are capable of getting the antibodies to their target cells in the first place, but they will also have an opportunity to tackle "the undruggable proteome," the massive roster of proteins that small-molecule drugs are currently unable to inhibit.

"It has been estimated that more than 80 percent of intracellular proteins are not druggable with traditional small-molecule drugs," Tsourkas says, "So, we believe that our technique gives us access to a wide range of new therapeutic targets."

More information: Hejia Henry Wang et al. Cytosolic delivery of inhibitory antibodies with cationic lipids, *Proceedings of the National Academy of Sciences* (2019). DOI: 10.1073/pnas.1913973116

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