Team reveals never-before-seen antibody binding, informing liver cancer, antibody design

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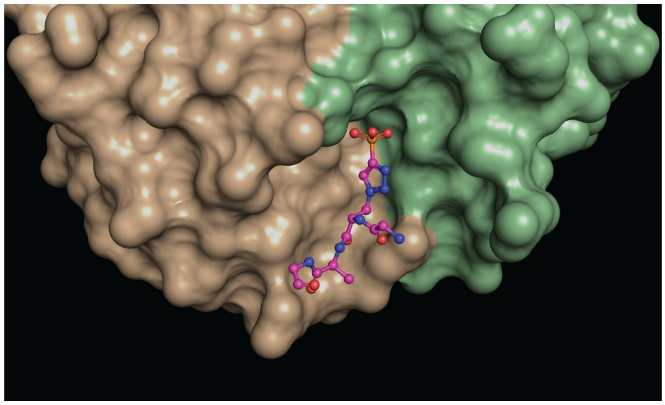

Caption: A structural snapshot of a phosphohistidine analogue (ball and stick model) nestled at the interface between different areas (green, brown) of a phosphohistidine antibody. Such structures provide insights into the molecular properties of the antibodies, which makes them useful for revealing elusive phosphohistidine-containing proteins in cells. Credit: Salk Institute

In structural biology, some molecules are so unusual they can only be captured with a unique set of tools. That's precisely how a multi-institutional research team led by Salk scientists defined how antibodies can recognize a compound called phosphohistidine—a highly unstable molecule that has been found to play a central role in some forms of cancer, such as liver and breast cancer and neuroblastoma.

These insights not only set up the researchers for more advanced studies on phosphohistidine and its potential role in cancer, but will also enable scientists to manipulate the shape and atomic makeup of the antibodies' binding sites in order to design ever more efficient antibodies in the future. The study was published in the *Proceedings of the National Academy of Sciences* on February 5.

"We are excited that these new antibody structures reveal novel principles of antigen binding. Now we can redesign these antibodies and engineer their properties to make them more efficient," says Tony Hunter, Renato Dulbecco Chair and American Cancer Society Professor at Salk and the paper's senior author. "This work may also provide other scientists with phosphohistidine antibodies that better suit their research purposes."

Amino acids are joined together in precise sequences to form proteins, and several of them can undergo chemical transformations that can change the activity of the protein for better or worse. One such transformation is a process called phosphorylation, when a compound called phosphate is added to an amino acid, changing its shape and charge. Previously, Hunter showed that phosphorylation on the amino acid tyrosine can drive cancer progression, a discovery that led to numerous anticancer drugs. More recently, Hunter turned his attention to phosphorylation of the amino acid histidine (which creates phosphohistidine), suspecting that the process might also play a role in human disease.

Hunter developed a suite of antibodies able to bind to phosphohistidine in proteins, and used chemically stabilized phosphohistidine analogues to develop a series of monoclonal antibodies that could recognize these forms. The next step was to understand exactly how the antibodies are able to bind to phosphohistidine. This led Hunter to collaborate with Ian Wilson, the Hansen Professor of Structural Biology at the Scripps Research Institute and a world-renowned expert in using protein crystallography to define antibody structures, to study the structures of the phosphohistidine antibodies.

"My long-term colleague Tony and I have been collaborating on this project for the past seven years," says Wilson. "We have obtained new insights into how antibodies can evolve to recognize phosphates linked to proteins, which is very satisfying."

To find out how phosphohistidine is recognized, they needed to image their antibodies in the act of binding the phosphohistidine, and so formed crystals between each antibody bound to a phosphohistidine peptide.

"To understand the molecular interactions between the antibodies and phosphohistidine, we needed to look at them in great detail," says first author Rajasree Kalagiri, a Salk postdoctoral researcher and expert in X-ray crystallography. "Once we got the antibodies to form crystals, we bombarded those crystals with X-rays to obtain a diffraction pattern. We then applied methods that transform the diffraction pattern into a three-dimensional electron density map, which was then used to discern the atomic structure of the antibodies."

The two types of antibody crystal structures solved by the team revealed exactly how different amino acids are arranged around the phosphohistidine to bind it tightly. Their five structures more than double the number of phospho-specific antibody structures previously reported, and provide insights into how antibodies recognize both the phosphate and the linked histidine. They also reveal at a structural level how the two types of antibody recognize different forms of phosphohistidine, and this will allow the scientists to engineer improved antibodies in the future.

More information: Rajasree Kalagiri et al, Structural basis for differential recognition of phosphohistidine-containing peptides by 1-pHis and 3-pHis monoclonal antibodies, *Proceedings of the National Academy of Sciences* (2021). DOI: 10.1073/pnas.2010644118

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