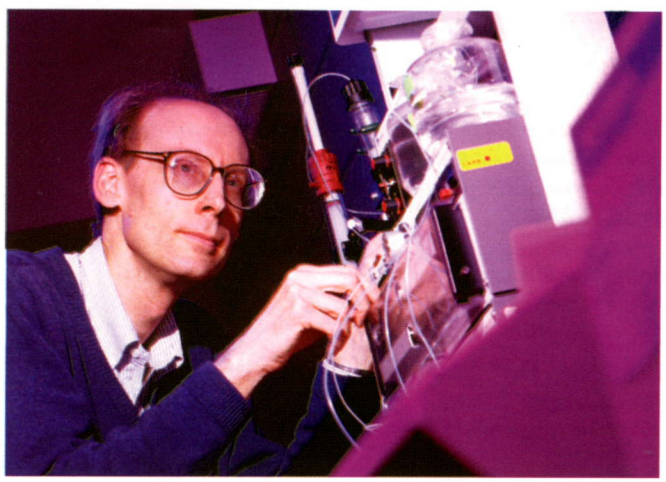
Watching Antibodies Get a Grip

As critical components of the immune system, antibodies play a lethal game of tag with foreign substances, or antigens, flagging them for destruction. On first encounter with an alien molecule, antibody-producing immune cells known as B lymphocytes deploy a wide variety of antibodies, only some of which have the proper shape to bind tightly to the antigen. Cells that generate the best-fitting antibodies proliferate, while cells that produce less-successful antibodies die off. With repeated exposure to the antigen, B lymphocytes continuously evolve to produce even tighter-gripping antibodies. Thus the immune system hones its defenses by fraternizing with the enemy. Despite extensive study, scientists have lacked details about how this process progresses at the molecular level.

Now Dr. Roy A. Mariuzza, professor of biochemistry at the University of Maryland Biotechnology Institute in Rockville, Maryland, and his colleagues have captured the first high-resolution, three-dimensional snapshots of maturing antibodies as they perfect their ability to latch onto a specific foreign protein. Their findings lend insight into the evolution of protein-protein interactions and also may help to streamline the development of monoclonal antibodies, which are used increasingly as medications for a wide range of disorders, from cancer to allergies. The research included collaborative work with Dr. Sandra J. Smith-Gill, who isolated the antibodies at the National Cancer Institute in Frederick, Maryland.



Dr. Roy Mariuzza's structural studies of antigen-antibody pairs showed how antibodies evolve to gain a tighter hold onto foreign molecules. *(Photo courtesy of Dr. Mariuzza)*

To effectively grasp a foreign protein, the molecular shape of an antibody and an antigen must be precisely aligned, much like the oft-used lock-and-key analogy. That careful alignment can be achieved in a matter of weeks or months, driven by spontaneous mutations as B lymphocytes reproduce and multiply. Offspring cells that generate better-fitting antibodies become even more prolific, spawning daughter B cells with slight mutations that may produce even tighter-gripping antibodies. Over time, these cellular mutations collectively fine-tune the antibodies' shapes to perfectly match a specific antigen. This process of molecular evolution, called affinity maturation, often enhances an antibody's binding ability by 100-fold during an immune response.

To get a picture of the hidden mechanics of affinity maturation, Dr. Mariuzza's research team examined crystals of four different antibodies, each clutching the same antigen, in successive stages of affinity maturation. In contrast, the few crystallographic studies of affinity maturation performed in the past all analyzed antibodies bound to small molecules, even though most biological antigens are large proteins.

The scientists' detailed molecular analyses depended on the use of high-intensity X-rays available at the NCRR-supported Resource for Macromolecular Crystallography at the National Synchrotron Light Source, Brookhaven National Laboratory, in Long Island, New York. The resource, headed by Dr. Robert Sweet, develops new technologies and research methods and provides scientists with access to five synchrotron beamlines, each equipped with state-of-the-art X-ray detectors and other advanced instrumentation.

By exposing crystallized samples of antigen-antibody pairs to beams of synchrotron radiation, and then analyzing the patterns produced by the diffracted X-rays, Dr. Mariuzza and his colleagues were able to determine the three-dimensional structures of the molecules. The synchrotron-derived images had such high resolution that they revealed extremely slight differences in the alignment of the antibodies—variations that were only several atoms wide.

When the scientists compared the computer-generated images of the four antigen-antibody pairs, they could clearly see how the antibodies evolved during affinity maturation. What they found were not dramatic structural transformations, but rather minor modifications. The researchers discovered that a mere handful of amino acid substitutions in the antibody improved its fit to the antigen. "The structure of the antibody basically adjusts over time so that it fits better against the antigen. Imperfections are eliminated at the

interface," says Dr. Mariuzza. "These imperfections can be holes or cavities where things don't fit together quite well, or maybe atoms that are clashing because they are too close."

These structural changes collectively foster another major change that draws the antibody closer to the antigen—a progressive increase in the binding regions that are hydrophobic, or repelled by water. Like two drops of oil that quickly unite when dropped into a bucket of water, the hydrophobic regions of the antigen and antibody are promptly drawn to each other.

Surprisingly, the changes that occurred in the antibodies over time were not made in the binding regions, or "hot spots," in the central core of the antibody. Instead, these changes occurred in areas that surround the hot spots. "You might think that a way to improve affinity would be to make more hot spots in the center, or to make existing hot spots even hotter. But that's not what we saw," says Dr. Mariuzza. "We think that's because the central core of the antibody is about as good as it can be—it's already been optimized. To improve beyond that, changes must be engineered elsewhere in the interface, which is why affinity maturation proceeds in peripheral regions that don't quite fit together yet."

Dr. Mariuzza suspects that his findings can be generalized to explain the affinity maturation of most antibodies, and therefore might provide insights into engineering monoclonal antibodies for use as therapeutics. Monoclonal antibodies are homogenous antibodies produced by fusing a single B lymphocyte clone with tumor cells, thereby generating cells that will proliferate indefinitely and produce relatively large amounts of a single type of antibody. Currently, drug companies try to mimic natural affinity maturation by inducing random changes in antibodies until they find one that fits snugly with the antigen of interest. But Dr. Mariuzza's findings suggest more targeted approaches to improving antibody affinity, such as inducing changes only on the portions of the antibody surrounding the central binding regions.

Dr. Mariuzza began working on antibody maturation by collaborating with Dr. Cesar Milstein, who won the 1984 Nobel Prize in Physiology or Medicine, along with Dr. Georges Köhler, for developing the first monoclonal antibodies. Their technique revolutionized medical diagnostics, which uses monoclonal antibodies in tests for a range of conditions, from strep throat to pregnancy. Monoclonal antibodies also are used as new drugs for breast cancer, non-Hodgkin's lymphoma, and asthma. So it is only fitting that Dr. Mariuzza's investigations, which began in collaboration with the father of monoclonal antibodies, should offer a way to improve their development in therapeutics.

Dr. Mariuzza's findings go beyond the realm of immunology, as they provide more general insights into how proteins bind to each other. Protein-protein binding underlies many physiological activities, from the actions of hormones to the spread of cancer cells. "There's a lot of interest in designing compounds that block protein-protein binding," he says. "Designing a molecule that prevents a hormone binding to its receptor, for example, can have important pharmaceutical implications." Such studies have been difficult to conduct in the past because of limited understanding of the factors that drive protein-protein recognition. But as more investigators uncover the chemistry of protein-protein attraction, says Dr. Mariuzza, bioengineers may be able to develop a whole new class of medicines.

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—Margie Patlak

For more information about the NCRR-supported Resource for Macromolecular Crystallography at the National Synchrotron Light Source, visit www.px.nsls.bnl.gov. For more information about other NCRR-supported synchrotron resources, visit www.ncrr.nih.gov/ncrrprog/btdir/Synchron.asp.

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Additional Reading

1. Li, Y., Li, H., Yang, F., et al., X-ray snapshots of the maturation of an antibody response to a protein antigen. *Nature Structural Biology* 10:482-488, 2003.
2. Sundberg, E. J., Andersen, P. S., Schlievert, P. M., et al., Structural, energetic, and functional analysis of a protein-protein interface at distinct stages of affinity maturation. *Structure* 11:1151-1161, 2003.