BY MARCUS WOO

PROTEINS FOR EVERY OCCASION

Protein design ascends to new heights

Scientists are now able to design, in principle, almost any protein they want—a feat that was inconceivable just a few years ago. They are reengineering existing proteins found in nature, as well as constructing proteins from the ground up, atom by atom.

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Designed proteins may help solve many of the world's biggest problems, says **David Baker, PhD**. As a professor of biochemistry at the University of Washington in Seattle, he's been a pioneer in developing computational methods to build proteins from scratch, a process called *de novo* protein design.

Thanks to an improved understanding of how proteins fold, as well as advances in computing and genomic technology, experts say the field is now at an inflection point, with progress developing faster than ever.

"I'd like to think we can do most everything," says **William DeGrado, PhD**, professor of pharmaceutical chemistry at the University of California, San Francisco. "And I'd like to think we can do a lot more than nature can do."

Designing a Protein

Scientists have understood the basic principles behind protein folding since the 1960s: Electrostatic forces between and among the amino acids in a protein sequence pinch the chain, folding it into its lowest energy state—a flexible 3-D structure that changes in response to other nearby molecules. Since then, progress toward understanding how proteins reach their 3-D structures has been steady, including the first *de novo* computational design of a protein nearly 20 years ago and many other protein design successes since.

These days, researchers can fully model and create proteins from scratch using an advanced software package called Rosetta, developed by Baker's lab. Rosetta users start with a desired protein structure and allow the program to fill in the details. Specifically, users first define a desired backbone shape—the arrangement of alternating amino and carboxyl groups that are part of each amino acid and that link together to form a polypeptide chain. The computer then calculates how well various side chains (which differ for each amino acid) fit around that backbone to produce the desired structure. "If you put a side chain in one position, that can dictate what's on the neighboring position so they nestle together," says **Brian Kuhlman, PhD**, professor of biochemistry and biophysics at the University of North Carolina at Chapel Hill. Meanwhile, Rosetta ensures that the whole molecule is at its lowest, most stable, energy state.

Yet most of the sequences that Rosetta comes up with for a particular structure won't actually fold into a stable shape in the lab. And calculating backward to check whether the sequences do indeed generate the desired protein structure only gets you so far. To truly validate a sequence, one must synthesize the protein and test its stability. Until the advent of large-scale *de novo* approaches (see below), this required that proteins be designed and tested one at a time.

Neanderthal Design: Tweaking Natural Proteins

Most protein engineering to date has involved tweaking proteins found in nature to give them slightly different functions. Baker calls this Neanderthal protein design, similar to the strategy our primitive cousins would have employed—fashioning tools out of what was already lying around—for example, chipping away at a rock or sharpening a stick.

Baker's team published an exciting example of this strategy in *Nature Biotechnology* in June 2017: They designed a protein that prevents mice from getting the flu. They knew that the flu virus's surface contains a mushroom-shaped protein called hemagglutinin that enables the virus to infect cells by binding to a sugar molecule in the cell membrane. So they created a protein, dubbed flu glue, that can glom onto hemagglutinin, blocking it from infecting cells. It might not become medicine for humans anytime soon, but could

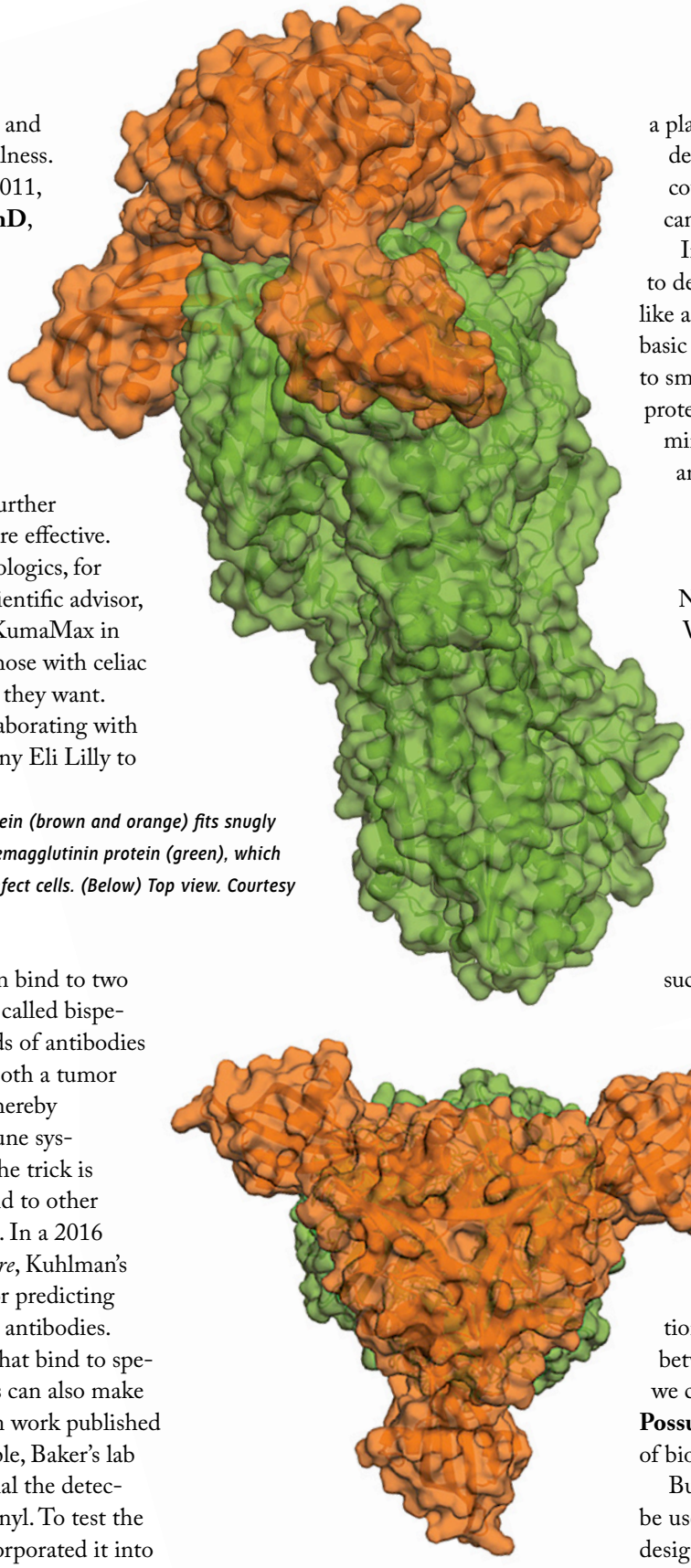
be used to develop a quick and easy way to diagnose the illness.

A few years earlier, in 2011, **Ingrid Swanson Pultz, PhD**, translational investigator at the University of Washington, led the development of an enzyme called KumaMax that breaks down gluten. Since then, the molecule's design has gone through further refinements to make it more effective. Pultz co-founded PVP Biologics, for which Baker serves as a scientific advisor, to further commercialize KumaMax in pill form. It might allow those with celiac disease to eat all the bread they want.

Kuhlman has been collaborating with the pharmaceutical company Eli Lilly to develop antibodies that can bind to two antigens at the same time, called bispecific antibodies. These kinds of antibodies can, for example, bind to both a tumor cell and an immune cell, thereby recruiting the body's immune system to help fight cancer. The trick is making sure they don't bind to other things in undesirable ways. In a 2016 paper published in *Structure*, Kuhlman's lab developed a strategy for predicting the specificity of bispecific antibodies.

By designing proteins that bind to specific molecules, researchers can also make new types of biosensors. In work published in *eLife* in 2017, for example, Baker's lab designed one that can signal the detection of the painkiller fentanyl. To test the sensor, the researchers incorporated it into a plant so the leaves turn color when it detects the molecule in question. This could ultimately lead to plants that can sense dangerous compounds.

In the future, Baker also wants to design proteins that can function like a rudimentary computer that does basic logic operations. This could lead to smart therapeutics such as designer proteins that can bind to a cell, determine whether it's healthy or sick, and release or not release a drug.



Flu glue: (Right) A designer protein (brown and orange) fits snugly on top of the influenza virus's hemagglutinin protein (green), which helps the virus latch onto and infect cells. (Below) Top view. Courtesy of Eva-Maria Strauch.

De Novo Design of Simple Proteins

Neanderthal design has its limits: When you start with protein backbones that were created through the evolutionary process, you miss out on a huge variety of options that nature never tried. By contrast, *de novo* design can explore the entire realm of possible protein backbones, some of which might have greater potential to prevent or treat disease than natural (or Neanderthal-designed) molecules such as antibodies or antibiotics.

For the most part, *de novo* efforts have been restricted to simpler proteins because more complex structures are beyond current computational capabilities. And researchers are still far from being able to design proteins with the same sophisticated functions as those in nature. "The gap between what nature can do and what we can build is still very wide," says **Possu Huang, PhD**, assistant professor of bioengineering at Stanford University.

But simpler proteins can still be useful. Huang, for example, has designed a donut-shaped protein called