ALS SCIENCE HIGHLIGHT

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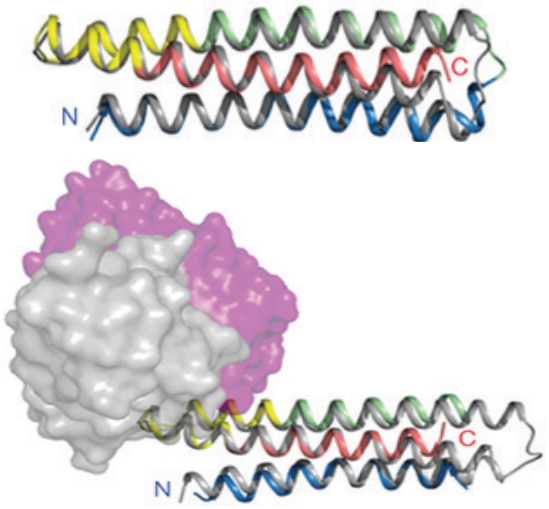
Validating Computer-Designed Proteins for Vaccines

In the struggle to keep up with microbes whose rapid mutations outpace our ability to produce vaccines, the human race has a powerful ally: computers. Researchers have now figured out a way to use computational protein design to generate small, stable proteins that accurately mimic key viral structures; these can then be used in vaccines to induce potent neutralizing antibodies. The results were validated in part using protein structures obtained at several of the Berkeley Center for Structural Biology protein crystallography beamlines at the ALS. The results have yielded promising leads for the development of vaccines to protect infants, young children, and the elderly against respiratory illness. More generally, the results provide proof of principle for this approach, which can apply to a variety of other vaccine targets, such as human immunodeficiency virus and influenza.



Top: Crystal structure of one of the designed proteins (FFL_005), superimposed in gray with the design model. Bottom: Crystal structure of a different designed protein (FFL_001) bound to an antibody, also superimposed with the design model in gray.

The researchers in this study used the fact that there are patches on pathogens that antibodies recognize: the "fingerprints," or in biological terms, the "epitopes." Using this new method called "epitope-focused vaccine design," the scientists first isolated antibodies from patients infected with RSV (respiratory syncytial virus), a virus resistant to current strategies for designing vaccines and that is responsible for an estimated annual six to seven percent of deaths of children under age one. Then, working backward from those antibodies, they determined the epitope that the antibodies recognize. It was important in this case to use a class of antibodies called "broadly neutralizing," which means that they recognize a key epitope on the virus—a region that the antibodies recognize no matter what mutations might occur in other regions of the pathogen. Often, viruses hide these key epitopes from detection by antibodies, which is part of the reason it is difficult to design vaccines against them.

The researchers then took that epitope, the unchanging fingerprint of the virus, and computationally modeled it into various protein scaffolds, designing a virtual virus. Computationally designing a protein is no easy task: for many

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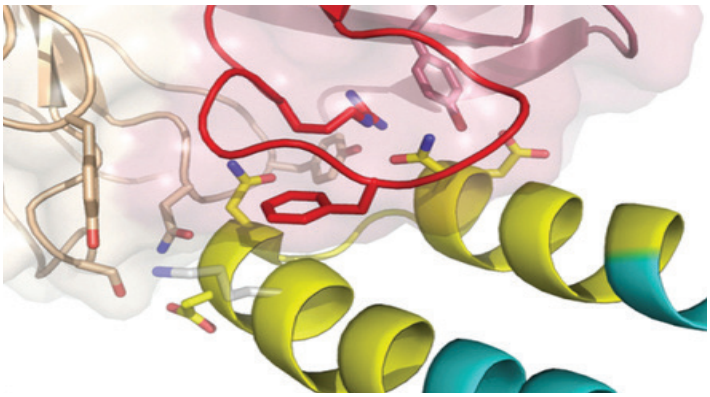
Wanted: Dead or Computed

As strange as it sounds, most vaccines are composed of actual dead viruses and bacteria. The idea is that presenting a dead form of the pathogen will fake your body into mounting an immune response, in which antibodies against those invaders are made and held in store for when the real attack occurs. And generally this works. Our immune systems, after all, are highly specialized and efficient producers of antibodies, those proteins that identify and kill the pathogens that are constantly attacking us throughout our lives.

But what do you do if you can't design a vaccine that elicits the proper immune response? Or what if those wily invaders mutate so fast that vaccine makers struggle to identify those invaders, let alone produce vaccines composed of dead forms of those invaders? This is the case in several major pathogens, such as HIV and influenza. What we need is a different way to seduce the immune system into producing the correct antibodies before the attack occurs. One ingenious idea, introduced by the Schief and Baker labs in recent years, is to computationally design an artificial pathogen rather than using the real ones. Their results, validated at the ALS, encourage the further development of these strategies for a variety of other vaccine targets.



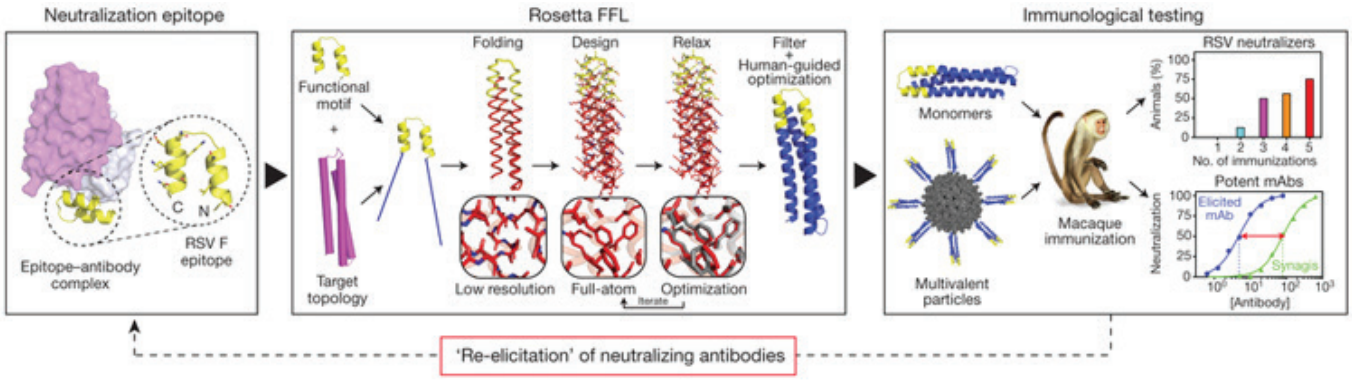
Close-up view of the interface between an antibody variant (17-HD9) and epitope.

years researchers have tried to design proteins *de novo*, but when the designed proteins are grown in cells, they often lack functional ability. The scientists in this study used a new approach in that they allowed much greater flexibility in their scaffold protein structure, allowing the computationally modeled protein to "virtually" fold in many different ways around the epitope. They then used energy-minimizing algorithms to select the most stable structures.

This computationally intensive approach paid off. When the resulting structures were expressed in and purified from *E. coli* cells, they behaved like normal proteins with good functional activity, and even better, they showed excellent affinity for the human antibodies for which they were designed. Finally, testing in rhesus macaques showed that the animals produced antibodies in response to the designed viruses.

But did these computationally designed proteins really grow into the structures that the researchers had designed? This is where protein crystallography comes in; crystallography is the foremost technique to look at protein structure on an atomic level. The designed proteins were crystallized and the structures determined at ALS Beamlines 5.0.1, 5.0.2, and 8.2.2, which not only gave the final proof that the proteins were exactly what the researchers had designed, but also gave deeper insight into why and how they bound to the antibodies.

This new method—computationally designing a "wanted poster" for the immune system—promises to open up a whole new way of making vaccines. Designed with sophisticated computational routines, grown in a lab, and then structurally determined using crystallography, the designed pathogen can mimic a real pathogen well enough that the body is tricked into producing protective antibodies, so that when and if the real pathogen shows up, the immune system is ready. The days of using dead viruses against themselves may soon be over.



Stages of computational design and immunological evaluation, from identifying the target epitope, to computational design, to testing. The computational procedure (the middle section), is called Fold From Loops (FFL) and has four stages: (1) selection of the functional motif and target topology to be folded around the motif; (2) ab initio folding to build diverse backbone conformations consistent with the target topology; (3) iterative sequence design and structural relaxation to select low-energy amino-acid sequences for the given backbone conformations; (4) filtering and human-guided optimization.