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Crystallizing a New Approach to Protein Structure

To better understand a protein's function, researchers often use a technique called x-ray crystallography to determine the three-dimensional architecture of the protein of interest. One of the most challenging aspects of this work, however, is that proteins can be notoriously difficult to crystallize. Protein crystals are the starting point for x-ray crystallography studies.

Now, Howard Hughes Medical Institute researchers and their colleagues have developed a systematic method for speeding up the crystallization of proteins, an advance that may greatly aid x-ray crystallography. The new technique analyzes the solubility of minute amounts of protein in a range of solvents and conditions. With that information in hand, researchers can generate phase diagrams for proteins and create customized crystallization recipes for each protein.

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— **Stephen R. Quake**

Howard Hughes Medical Institute investigator Stephen R. Quake and his colleagues described the new method in an article published on November 7, 2006, in the *Proceedings of the National Academy of Sciences*. Quake is at Stanford University. Quake's co-authors included first author Megan J. Anderson of Stanford and the California Institute of Technology and Carl L. Hansen of the University of British Columbia.

Proteins are biological machines, and scientists need to determine their structures to understand how the proteins work. The most widely used method for structure determination is x-ray crystallography. In that technique, crystallized proteins are first bombarded with x-ray beams. As the x-rays pass through and bounce off of atoms in the crystal, they leave a diffraction

pattern, which can then be analyzed to determine the three-dimensional shape of the protein.

Crystallizing proteins has become one of the main bottlenecks in scientists' efforts to develop large-scale approaches to determine protein structures more rapidly, said Quake. Part of the reason is that other steps have gotten easier, he said. In particular, there are very powerful computational tools that let you, once you have a good diffraction pattern, recover the atomic coordinates of the atoms in the proteins. Also, he said, the processes of purifying proteins have become more sophisticated and automated.

But getting the crystals is tricky because there has been very little ability to predict the conditions required for crystallization of a given protein, he said. Each protein is different, so crystallizing each one is a unique problem. Thus, investigators have been reduced to using a brute force approach, in which they randomly screen a bunch of conditions that may or may not work, and then try to deduce the optimal conditions necessary for crystallization, he said.

The new approach to designing a crystallization strategy is based on a microfluidic device called a formulator that was developed by Hansen in Quake's laboratory. The formulator consists of a small central mixing chamber fed by 33 tiny channels. The researchers can program the formulator to feed a precise recipe of chemicals into the mixing chamber and observe the resulting solubility of the protein sample in the chamber. The device works with volumes measured in nanoliters, or one billionth of a liter.

In the new study, the researchers chose to test their technique on 12 proteins submitted by other scientists that had not yet been crystallized. The proteins represented a range of sizes and biological functions. Anderson used the formulator to survey the solubility of these proteins in a variety of reagents. The reagents she used consisted of a range of polyethylene glycol molecules of different polymer chain lengths and chemically modified chain ends. She also varied conditions such as the acidity of the solution.

After Anderson measured each protein's solubility under a range of conditions, she constructed a phase diagram for the protein and a given solvent. A phase diagram depicts the protein's solubility at different protein and reagent concentrations. The phase diagrams yielded data that enabled the researchers to choose an optimal solvent and conditions for crystallization of a given protein.

Anderson next used the phase diagrams to design crystallization conditions for six proteins and crystallized each of those proteins. Those crystals were then tested to evaluate their diffraction quality. The researchers achieved a 75 percent success rate in producing protein crystals, which is roughly double the overall success rate in producing such crystals, said Quake. They achieved diffraction-quality crystals in a third of the cases, which was also significantly greater than obtained by the usual methods.

Unfortunately none of the crystals were ordered enough to solve a protein's structure, he said. That's not surprising, given that we didn't have any control over the purification of the proteins; nor did we do the kind of 'editing' of the protein molecule that investigators usually do to optimize crystallization. So, in fact, it was more surprising that we got any diffraction patterns at all with these random samples.

Quake said his next goal is to demonstrate the usefulness of their systematic crystallization approach in structure determination by taking a range of proteins through the entire process from purification to x-ray crystallography.

Quake said that he and his colleagues are making their technique widely available to researchers. They are constructing the microfluidic formulators for other laboratories and are offering a course to train researchers in the technology.