Duke technique is turning proteins into glass

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Duke University researchers have devised a method to dry and preserve proteins in a glassified form that seems to retain the molecules' properties as workhorses of biology.

They are exploring whether their glassification technique could bring about protein-based drugs that are cheaper to make and easier to deliver than current techniques which render proteins into freeze dried powders to preserve them.

Duke engineer and chemist David Needham describes this glassification process as "molecular water surgery" because it removes virtually all the water from around a dissolved protein by almost magically pulling the water into a second solvent.

"It's like a sponge sucking water off a counter," said Needham, a professor of mechanical engineering and materials science at Duke's Pratt School of Engineering, who has formed a company called Biogyali ("gyali" means glass in Greek) to develop the innovation. That firm has also applied to patent the idea of turning proteins into tiny glass beads at room temperature for drug delivery systems.

A report by Needham, graduate student Deborah Rickard and former graduate student P. Brent Duncan online in the *Biophysical Journal* describes how his team carefully controlled water removal during glassification by releasing single tiny droplets of water-dissolved protein into the organic solvent decanol with a micropipette.

Preliminary evaluations by his senior scientist David Gaul and a team of undergraduate students showed that four test proteins undergoing such procedures retained all or most of their original activity when water was restored. His group has received about $1 million from the National Institutes of Health grants for the research.

Having devised a way to turn proteins into glassy microbeads measuring only about 26 millionths of a meter in diameter, Needham hopes those can be directly injected into the body for use as "biologic" drugs.

His group's early research shows high concentrations of such tiny beadlets would not be as viscous as proteins dehydrated into the normal powder form, which tend to clog up syringes, he said.

These microbeads might also be packaged for slow time-release by surrounding them with a polymer that would biodegrade over time, though how to do that has not been resolved yet, he added.

In collaborations with Duke's Brain Tumor Center and Comprehensive Cancer Center, the researchers are seeking additional funding to do initial evaluations on glassified forms of three molecules with drug potential.

One, known as O6-AMBG, can help the cancer drug Temozolomide work better when infused into brain tumors. A second, Lapatinib, is designed to knock out other molecules that help cancer cells grow in the breast and elsewhere. The third, shepherdin, also targets breast cancers.

Their discovery of protein glassification grew out of a basic exploration of a general question: What can dissolve in what?

Needham's research group found, for example, that air and the organic liquid chloroform will both dissolve in water at about the same rate. It

also found that water will dissolve in decanol, a substance it cannot even mix with in large quantities.

These experiments, and the theory underlying them, are described in a second report led by Needhams's graduate student Jonathan Su, now published online in the *Journal of Chemical Physics* (http://link.aip.org/link/?JCP/132/044506).

"Mixing" and "dissolving" are not the same thing, Needham said. "A good example of a suspended mixture is salad dressing, where oil and water are mixed but oil does not appreciably dissolve in water, nor water in oil."

They next tried a more complex variation of a familiar high school experiment which dissolves so much salt in water that some begins coming back out of the solution as a crystal.

In this case, after dissolving the salt in water, Needham's group then inserted a microbubble of that solution into immiscible decanol in a microscopic chamber. The water itself then dissolved into the decanol and left behind the salt, which also crystallized.

According to his group's Biophysical Journal report, while decanol has practically no tendency to dissolve in water, water has a high probability of dissolving in decanol, allowing the latter to be used as a "drying" agent to remove the former.

"So then we asked: what if we did the same thing with the protein albumin?" Needham said. "I expected to maybe get crystallized albumin," Needham recalled. "But, in just a few minutes, we instead formed a glassified microbead of protein on the tip of a micropipette, at a high density just a bit more dense than water itself. That protein glass is not a crystal. It's really a solid liquid."

Many proteins can be coaxed into forming crystals, solids created by repeating three dimensional patterns of atoms as surrounding water is removed. On the other hand, Needham said he was not really surprised that his protein samples instead formed into glasses, which are more unorganized assemblage of molecules that can still "flow" over very long time scales.

The water loss in his process is apparently too rapid for the molecules of big and irregular proteins to reorganize into a crystal form in such a short time, he explained.

Careful studies by his graduate student Rickard found that the decanol removed all the water that is not bound up in the proteins' molecular structures. And the remaining "bound" water was insufficient to support the growth of bacteria and fungi. Storing proteins as microbeads could thus preserve them.

Proteins are currently dried into clumpy, irregular powders by several industrial processes -- usually freeze-drying -- to protect them from such microbe damage. Drying also avoids the chemical breakdowns that can also occur when proteins are kept in solution. "But in the freeze-drying process itself, some very sensitive biologic drugs can also get damaged," Needham said.

Freeze-drying proteins into solids is also slower and more expensive than glassifying them, he added. And the resulting "flaky" powder is harder to handle than glassified beads.

Glassification "is a fast process," said Gaul, a senior research scientist in Needham's lab. Unlike freeze-drying, "we can dry particles within minutes, if not seconds, and don't need any specialized equipment."

More information: "Hydration Potential of Lysozyme: Protein

Dehydration Using a Single Microparticle Technique," Deborah L. Rickard, P. Brent Duncan, and David Needham, *Biophysical Journal*: Volume 98, Issue 6, 17 March 2010, Pages 1075-1084.
doi:10.1016/j.bpj.2009.11.043