Synthetic llama antibodies rescue doomed proteins inside cells

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Columbia researchers have created a new technology using synthetic llama antibodies to prevent specific proteins from being destroyed inside cells. The approach could be used to treat dozens of diseases, including cystic fibrosis, that arise from the destruction of imperfect but still perfectly functional proteins.

In many genetic diseases, including cystic fibrosis, mutated proteins are

capable of performing their jobs but are tagged for destruction by the cell's quality control mechanisms.

"The situation is analogous to ugly fruit," says Henry Colecraft, Ph.D., the John C. Dalton Professor of Physiology & Cellular Biophysics, who led the research. "Shoppers reject fruit that doesn't look perfect, even though ugly fruit is just as nutritious. If mutated proteins in cystic fibrosis can escape the cell's quality control mechanisms, they work pretty well."

In the cell, proteins destined for destruction are marked with a small peptide called ubiquitin. Deubiquitinase enzymes (DUBs) can remove these tags, but simply increasing DUB activity would indiscriminately rescue all proteins in a cell marked for destruction, which would be harmful.

"A lot of proteins are destroyed by the cell for good reason," Colecraft says, "so a therapy needs to be selective."

That's when Colecraft and his graduate student, Scott Kanner, realized they could develop a solution that takes advantage of nanobodies—small antibodies produced naturally by llamas, camels, and alpacas that were discovered nearly 30 years ago. These small nanobodies bind their targets with exquisite specificity and retain this property inside cells, unlike regular antibodies.

The new technology—called engineered deubiquitinases or enDUBs for short—combines a synthetic nanobody that recognizes a specific protein with an enzyme that can rescue proteins tagged for destruction.

In a new paper in *Nature Methods*, the researchers tested two different enDUBs, one designed to rescue a protein mutated in cystic fibrosis and another designed to rescue a protein mutated in long QT syndrome, an

inherited heart disease that can cause arrhythmia and sudden death.

To build each enDUB, the researchers first had to find a nanobody that only recognizes and binds the target protein. Until recently, researchers had to inject their target proteins into llamas, camels, or alpacas and wait for the animal to generate such nanobodies. The Columbia researchers instead fished out binders from a synthetic yeast nanobody display library containing millions of unique nanobodies.

Once created, each enDUB was tested in cells that produced the mutated proteins.

In both cases, enDUBs prevented the destruction of the proteins, and the proteins migrated to their normal locations in the cell membrane where they performed their normal functions.

"In the case of one of the cystic fibrosis proteins we tested, we get a remarkable rescue, restoring protein levels in the cell membrane to about 50% of normal," Colecraft says. "If that happened in a patient, it would be transformative."

Though both diseases investigated in the study are caused by mutations in ion channel proteins, "the approach can be applied to any protein in the cell, not just membrane proteins or proteins altered by genetic mutations," Colecraft says.

"It could be applicable to any disease where protein degradation is a factor, including cancer and epilepsy."

More information: Scott A. Kanner et al, Targeted deubiquitination rescues distinct trafficking-deficient ion channelopathies, *Nature Methods* (2020). DOI: 10.1038/s41592-020-00992-6

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