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Changing the identity of cellular enzyme spawns new pathway

By Tom Fleischman

Integral membrane proteins, or IMPs, are a major class of proteins that play crucial roles in many cellular processes, including the catalysis of disulfide bonds, which are essential for the function and stability of many proteins such as antibodies, which have significant therapeutic potential.

But IMPs are intrinsically hydrophobic and thus have low solubility in watery environments. Their natural environment is within the lipid bilayer membrane of a cell, which makes it difficult to study their structure and function.

A previously reported method involving standard recombinant DNA techniques and some novel design principles enabled a team of Cornell chemical engineers to make large quantities of functional IMPs simply and inexpensively – all without the use of harsh chemicals or detergents, which are typically used today. That team, led by Matt DeLisa, the William L. Lewis Professor of Engineering in the Robert Frederick Smith School of Chemical and Biomolecular Engineering, has now used that protein engineering method to convert a membrane-bound enzyme into a water-soluble biocatalyst that functions directly in the aqueous inner cell.

"You can redesign these tricky proteins, making them water-soluble, and perhaps really surprisingly, they can continue to catalyze their natural biological reactions," said DeLisa, principal investigator for "A water-soluble DsbB variant that catalyzes disulfide-bond formation in vivo," published June 19 in Nature Chemical Biology.

"To our knowledge, this is the first example of creating a water-soluble IMP that retains its natural catalytic activity but does so in an entirely new cellular environment," DeLisa said. "And because it's a genetically engineered construct, it can be expressed like any other soluble protein with very little effort or difficulty."

First author is Dario Mizrachi, former postdoctoral associate in chemical and biomolecular engineering who's now an assistant professor at Brigham Young University. Collaborators included Michael-Paul Robinson, doctoral student in chemical and biomolecular engineering, and Mehmet Berkmen of New England Biolabs.

The group's previous work detailed a method they called SIMPLEx (Solubilization of Integral Membrane Proteins with High Levels of Expression), for shielding IMPs from water and enabling the production of large quantities of these difficult-to-make proteins. Using recombinant DNA techniques, they stitched together an artificial membrane protein with an identity crisis – one that maintains its biological function, but thinks it's soluble in water.

This latest work is the first application of that technique. The group used their identity-switched IMPs to make disulfide bonds, a type of post-translational modification that occurs in many proteins and influences nearly all aspects of normal cell biology and pathogenesis.

The group targeted the bacterial integral membrane enzyme DsbB, a central biocatalyst in disulfide bond formation, although DeLisa believes the technique is transferrable to myriad other membrane proteins.

Using the SIMPLEx method, the group converted membrane-bound DsbB into a water-soluble biocatalyst that could be readily expressed in the E. coli cytoplasm, where it spawned disulfide-bond formation in a range of protein targets.

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Disulfide bonds are key players in many therapeutic proteins, such as monoclonal antibodies. Many cancer drugs employ these molecules, which can mimic or enhance the immune system's attack on tumor cells.

The ability to take the catalyst out of the lipid membrane and put it in the cytoplasm, DeLisa said, allows scientists to make these antibodies in potentially more favorable locations in the cell.

"We could make this pathway in the cytoplasm … [or] we could move everything to a different subcellular compartment like the periplasm, or potentially take the entire pathway out of the cell and reconstitute it in a cell-free system," DeLisa said. "The point is, we create a tremendous amount of flexibility in terms of making these bonds by essentially turning a membrane protein into a soluble enzyme."

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Small-angle X-ray scanning analysis for this work was performed at the Cornell High Energy Synchrotron Source (CHESS), a high-intensity X-ray source supported by the NSF, and in the Macromolecular Diffraction at CHESS (MacCHESS) facility. This work also made use of the Cornell Center for Materials Research, which is also supported by the NSF.

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