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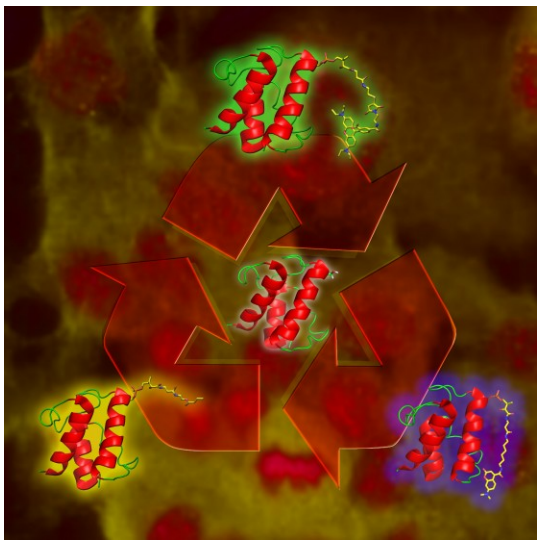
UC San Diego Chemists Develop Reversible Method of Tagging Proteins

Chemists at UC San Diego have developed a method that for the first time provides scientists the ability to attach chemical probes onto proteins and subsequently remove them in a repeatable cycle.



The new technique allows scientists to add and remove different kinds of chemical probes at specific locations on proteins, such as the fatty acid molecule shown here. Credit: J. LaClair, UC San Diego

Their achievement, detailed in a paper that appears online this week in the journal *Nature Methods*, will allow researchers to better understand the biochemistry of naturally formed proteins in order to create better antibiotics, anti-cancer drugs, biofuels, food crops and other natural products. It will also provide scientists with a new laboratory tool they can use to purify and track proteins in living cells.

The development was the culmination of a 10 year effort by researchers in the laboratory of Michael Burkart, a professor of chemistry and biochemistry, to establish a method to both attach a chemical probe at a specific location on a protein and selectively remove it.

This flexibility allows researchers to study the protein with many different functional attachments, providing versatility akin to a biochemical Swiss Army knife. The great advantage of this technique is the broad flexibility of the attachments, which can be dyes, purification agents or mimics of natural metabolic products. Each of these attachments can be used for different purposes and biological studies.

Burkart's goal in his own laboratory is to understand more about the biochemical pathways of fatty acid metabolism and the biosynthesis of other natural products. One project focuses on engineering algae in order to produce improved biofuels. In this effort, the scientists hope to

maximize the production of high quality algae oils, which could be used to supplement or supplant existing fossil fuels.

“In fatty acid metabolism, the fatty acids grow from an arm that eventually curls around and starts interacting with the metabolic protein,” said Burkart, who is also associate director of the San Diego Center for Algae Biotechnology, or SD-CAB, a consortium of institutions in the San Diego region working together to make biofuels from algae commercially viable as transportation fuels. “What we wanted to know was how long does the growing fatty acid get before it starts binding with the protein?”

Burkart and chemists in his laboratory—Nicolas Kosa, Robert Haushalter and Andrew Smith—found a way to remove the chemical probe from this metabolic protein using an enzyme called a phosphodiesterase derived from the common bacterium *Pseudomonas aeruginosa*. Subsequent reattachment of a fatty acid analogue reconstituted the protein complex to its natural state. By repeating the process again and again, while examining the molecular changes in the fatty acid with nuclear magnetic spectroscopy, or NMR, during different metabolic stages, the scientists were able to detail the biochemical pathway of the fatty acid metabolism in a way they had never been able to do before.

“Without this tool, we would really have very limited ways of studying the dynamics of these fundamental metabolic processes,” Burkart said. “This opened the door for us to finally examine in detail the fatty acid biosynthesis shared by algae, which you have to understand if you want to engineer ways to improve the quantity of oil that’s made by algae or to make different types of oil molecules in algae that are better for biofuels.”

The UC San Diego chemists also used NMR to verify that the process of chemically removing and attaching the chemical probes does not degrade or alter the protein in any way. “We’ve shown that we can do this iteratively, at least four or five times, without any degradation of the protein,” said Burkart. “The protein remains very stable and can be studied very easily.”

Because these same metabolic processes are shared by the metabolism of many natural products, including anti-cancer agents, antibiotics, and natural insecticides, Burkart said this new tool should have wide application in natural product chemistry labs.

“These are fundamental biochemical pathways that we still don’t fully understand,” he said. “We’re now learning how these basic biosynthetic enzymes work. A large majority of drugs are derived from natural products and many future medicines can result from these pathways. There’s a great interest now in synthetic biology, using these pathways to make new antibiotics or new anti-cancer drugs. They’re all regulated by these same types of interactions.”

The UC San Diego chemists say their method of tagging and removing chemical probes from proteins should also have wide application as a general laboratory tool to visualize and track proteins on living cells, as well as manipulate them outside of the cell.

“One could attach a tag, such as biotin, that would allow the protein to be purified. Then one can clip off the tag and attach a fluorescent molecule to monitor protein interaction with other molecular partners,” said Burkart. “The method could also be used for studying living cells, such as observing protein expression levels throughout the cellular life cycle. We certainly see that as a possible application.”

“Dr. Burkart’s new labeling technique gives scientists an unprecedented way to probe the complex catalytic machineries involved in the biosynthesis of natural products,” said Barbara Gerratana of the National Institutes of Health’s National Institute of General Medical Sciences, which partially funded the work. “The technology will help scientists harness these natural biochemical pathways to synthesize novel molecules for uses in a broad array of areas, including basic biomedical research and drug discovery.”

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