NEWS & ANALYSIS

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Scripps team develops technique for screening proteins

Researchers from The Scripps Institute (USA) have developed a method capable of detecting functional sites in proteins. The method could allow any research group take a set of cells and situate the active sites on their proteins to gauge the level of reactivity.

Currently, an amino acid sequence can be identified but the data do not demonstrate the reactive sites of a protein. This is due to the fact that the reactive sites are only evident once the amino acid chains have folded to form a 3D structure. It is possible to detect active sites using biochemical studies but this can often take months, or even years, for just one single protein.

The team, led by Benjamin Cravatt III, used cysteine as its target as a proof-of-principle. They developed two cysteine probes, which were chemically the same but which differed in mass, in order to be distinguished later. The probes were added to cellular proteins at one hundred-times lower than the concentration normally added in these types of study, so that most of the cysteines would be incompletely labelled. The concentration of the probes also varied so that one protein sample could be treated with up to ten-times more than another. The cysteines that were fully labelled could then be identified as the hyper-reactive cysteines.

This hypothesis was first tested on a human breast cancer cell line and 800 cysteine sites were identified in 522 proteins. A total of 90% of the cysteines in this sample showed the high- and low-level labeling, demonstrating that the cysteines had ordinary reactivity. The remaining 10% showed a constant level indicating that these cysteines were hyper-reactive.

This work was then followed up by a collaboration with researchers at the University of Washington (USA), who provided a set of synthetic proteins. These proteins had been designed specifically for enzymatic function. The test developed by Cravatt and his team was then used to identify which proteins may have the desired function.

Cravatt explains that, "our findings are quite significant for researchers interested in functionally annotating the proteome. Despite playing a critical role in many biochemical events such as enzyme catalysis and post-translational modification, the inherent reactivity of amino acid side chains has not, to date, been broadly profiled across the proteome. Thus, when one discovers new examples of hyperreactive cysteine residues in proteins, one can surmise that they likely contribute to the function of those proteins."

The team hopes to develop its method further because it has the potential to be applied to all the amino acids, as only the probe would need to be changed in each case. The group hopes that it has broad applications across biology and basic research and drug development. Speaking to *Future Medicinal Chemistry* Cravatt commented that "one of the advantages of our approach for enzyme design is that it provides quantitative information on the reactivity of active-site residues directly in native proteomes without requiring the purification of designed proteins. We are hopeful that our methods will help to define biochemical activities for poorly characterize proteins and, through doing so, illuminate new drug targets for diseases like cancer."

Source: Weerapana E, Wang C, Simon G *et al.* Quantitative reactivity profiling predicts functional cysteines in proteomes. *Nature* DOI:10.1038/nature09472 (2010) (Epub ahead of print).

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