PROTEOMICS

STOMPing at the bits

Spatially targeted optical microproteomics identifies novel amyloid plaque components.

Many neurodegenerative diseases, including Alzheimer's disease (AD), involve pathological protein deposits. It is known that these deposits are composed primarily of specific amyloid aggregates—Aβ protein in the case of AD—but there is growing interest in identifying their exact composition. Laser-capture microdissection followed by mass spectrometry has been used to characterize amyloid plaques. However, with a resolution of ~10 μm, the method is not ideal for analyzing the smaller inclusions typical of AD. To address this problem, Avijit Chakrabartty of the University of Toronto and colleagues have developed STOMP (spatially targeted optical microproteomics), an approach that combines two-photon laser-scanning microscopy with photochemical affinity labeling and mass spectrometry.

In STOMP, a fixed specimen is first stained with antibodies or fluorescent dyes to identify regions containing deposits. These areas are recorded as a digital mask file, and laser illumination is used to cross-link a six-histidine-conjugated photo-affinity tag in an automated fashion. Two-photon microscopy allows for a cross-linking volume smaller than 1 μm^3, enabling covalent attachment of the photo-tag to the target region with high precision. The sample is solubilized and the tagged proteins are purified by metal affinity prior to mass spectrometric identification.

To demonstrate STOMP's capabilities, Chakrabartty and colleagues analyzed the composition of amyloid plaques from an AD mouse model. As expected, the procedure identified a large amount of Aβ. In addition, 62 proteins were assigned a high probability of being associated with plaques, including SNAP25, VAMP2, synapsin 1 and ApoE, all of which were confirmed as plaque components by immunohistochemistry. Finally, the researchers demonstrated that STOMP can be used to analyze senile plaques from formalin-fixed postmortem AD brains. In both mouse and human tissues, the STOMP analyses showed enrichment of certain presynaptic proteins, providing evidence for an association between amyloid plaque formation and synaptic function.

Although the need to pool samples to obtain sufficient material for protein identification is a limitation, STOMP's spatial resolution suggests that it could be applicable to the analysis of other small cellular features not amenable to biochemical purification.

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RESEARCH PAPERS

Hadley, K.C. *et al.* Determining composition of micron-scale protein deposits in neurodegenerative disease by spatially targeted optical microproteomics. *eLife* doi:10.7554/eLife.09579 (29 September 2015).