PROTEOMICS

A MACnificent view of the cellular protein landscape

The multipurpose MAC-tag enables integrative interaction proteomics to localize proteins with sub-organelle precision.

Proteomics offers an array of strategies for mapping cellular protein interactions. Two popular methods are affinity-purification mass spectrometry (AP-MS) and mass spectrometry coupled with proximity-dependent biotinylation (BioID). Both AP-MS and BioID can identify partner proteins surrounding a tagged bait protein, but their scopes are somewhat different. Whereas AP-MS captures direct or indirect interaction partners with high specificity, BioID is able to identify the bait protein's entire 'neighborhood', represented by interacting and noninteracting proteins within a 10–50-nanometer radius. Integration of these approaches might allow for more comprehensive characterization of a protein's molecular environment but is quite laborious, as cell lines with different tags would need to be generated.

Markku Varjosalo's team at the University of Helsinki tackled this problem by designing a 'multiple approaches combined' (MAC)-tag that contains an affinity handle for AP-MS as well as the biotin ligase BirA* for BioID. Therefore, a single construct is sufficient for both analyses, and a researcher can readily switch from AP-MS to BioID by adding biotin to the cell culture media to activate BirA*.

The researchers applied the MAC-tag approach to 18 marker proteins with known subcellular localization. This provided a signature of interacting and proximal proteins for every cellular compartment. Alignment of this protein context map with the BioID profiles of other proteins directly revealed the subcellular localizations of these proteins.

Varjosalo and coworkers propose that MAC-tag-based protein context maps can be used for 'mass spectrometry–microscopy' to localize bait proteins with high spatial resolution. Indeed, the MAC-tag approach enabled them to define a sub-mitochondrial protein context map. Using this map for mass spectrometry–microscopy, they established the sub-mitochondrial localization of 13 proteins for which confocal microscopy could not distinguish distributions within the organelle. Zooming in even further, the AP-MS and BioID data also provide insights into the relative distances of subunits in large protein complexes such as transcription factor IIH and Mediator. Thus, the work of the Varjosalo team not only facilitates the integration of AP-MS and BioID, but also provides a resource for probing protein localizations at different levels of cellular organization.

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RESEARCH PAPERS

Liu, X. *et al.* An AP-MS- and BioID-compatible MAC-tag enables comprehensive mapping of protein interactions and subcellular localizations. *Nat. Commun.* **9**, 1188 (2018).