

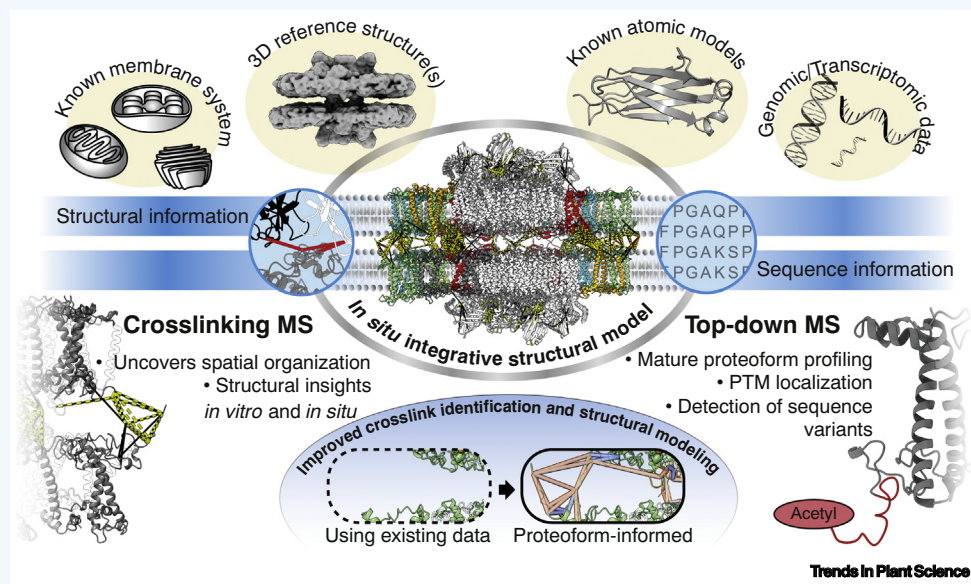
Structural Proteomics Applied to Plant Membrane Protein Complexes

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ADVANTAGES:

Analysis suitable for large membrane protein complexes, either detergent-solubilized form (*in vitro*) or embedded in the native membrane (*in situ*), provided the availability of genetic and 3D structural information.

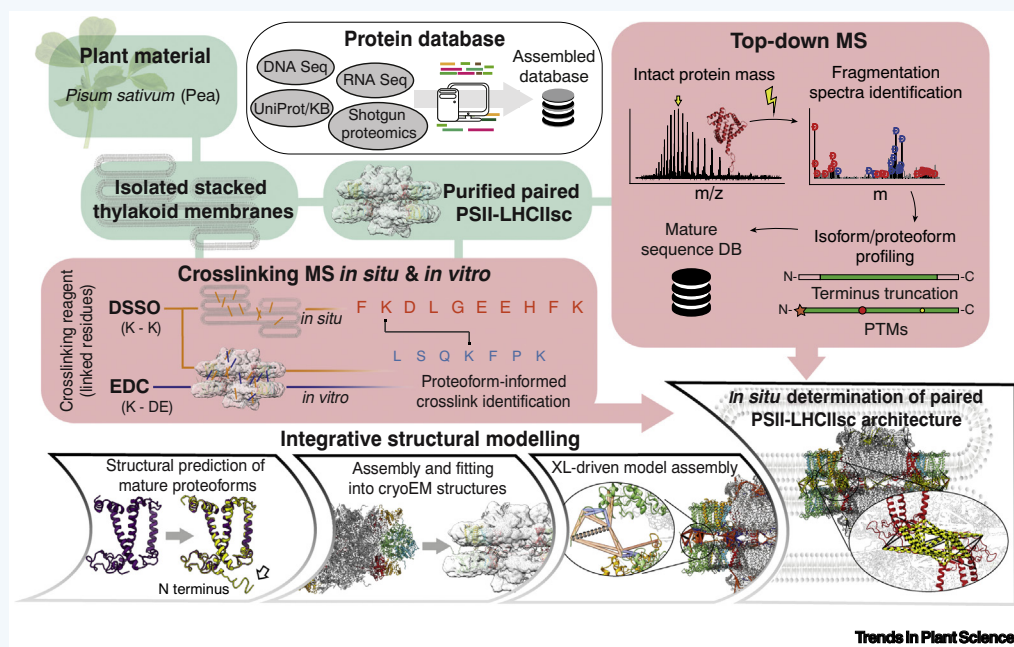
3D structures of large membrane protein complexes at intermediate resolution, achievable by cryoEM, are sufficient to reveal the overall organization.

TD-MS uncovers mature proteoforms, namely different forms of a protein arising from a given gene with a variety of sequence variants and PTMs. As such, it complements the public sequence databases by providing an exhaustive list of mature proteoforms.

XL-MS, informed by the TD-MS results, uncovers protein interactions and complements cryoEM results, providing protein localization within the 3D structure.

Integration of multiple tiers of structural information completes the picture of the overall membrane protein complex organization.

Membrane protein complexes are fundamental in many biological processes. Nevertheless, their structural details are difficult to resolve, especially in their cellular milieu. The combination of top-down (TD) mass spectrometry (MS), profiling post-translational modifications (PTMs) and sequence variants, and crosslinking (XL) MS for uncovering the spatial organization and interactors of protein complexes, provides a novel approach to study the structural behavior of protein complexes in their close to native environment.



CHALLENGES:

Initial availability of plant genetic information, at genomic or transcriptomic level, is required.

Protocols for sample isolation in close to native state need to be optimized for membrane protein complexes and native sourcing membranes.

It is difficult to confidently identify high molecular weight (≥ 100 kDa) proteoforms by TD-MS.

Efficient XL reagents for *in situ* XL-MS specifically target lysine amino acid residues, which have to be abundant and accessible in the target protein complex.

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In plants, grana-stack formation is still debated. By using TD-MS and XL-MS, *in vitro* on purified paired photosystem II–light-harvesting complex II (PSII-LHCII) supercomplexes (PSII-LHCIIsc) and *in situ* on their sourcing isolated stacked thylakoid membranes, we uncovered the spatial organization of paired PSII-LHCIIsc, revealing their role in grana stacking. Samples were isolated from pea, a plant for which transcriptomic data and a cryo-electron microscopy (cryoEM) 3D structure of paired PSII-LHCIIsc are available.

Acknowledgments

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