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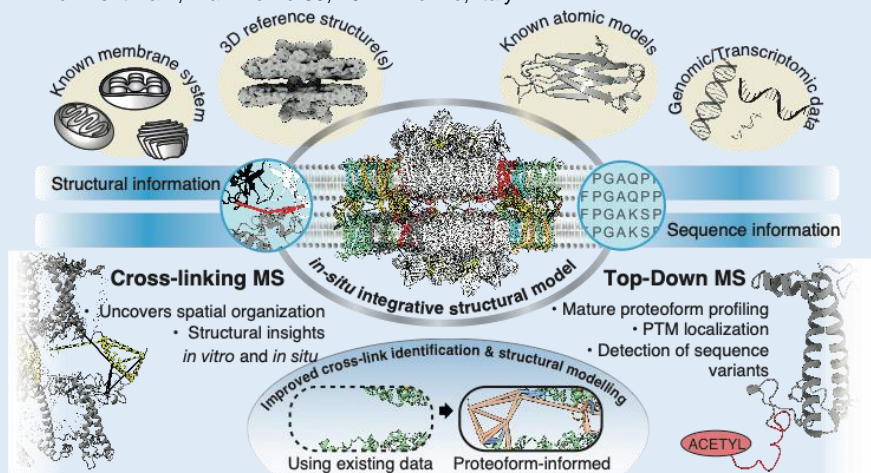
Structural proteomics applied to plant membrane protein complexes

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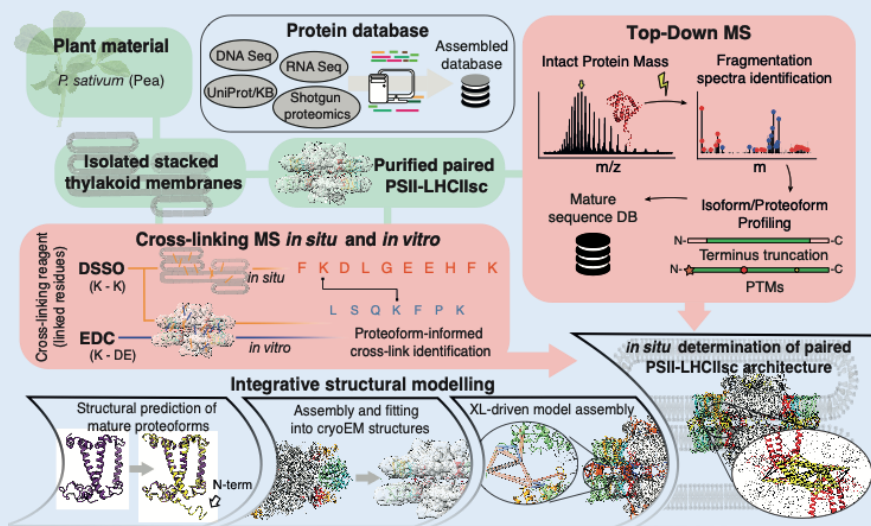
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Membrane protein complexes are fundamental in many biological processes. Nevertheless, their structural details are difficult to resolve, especially in their cellular milieu. The combination of top-down (TD) mass spectrometry (MS), profiling post translational modifications (PTMs) and sequence variants, and cross-linking (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.



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

Advantages:

Analysis suitable for large membrane protein complexes, either detergent-solubilized (*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 cryo-EM, 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 cryo-EM 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.

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 cross-linking 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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