TLP18.3 and Psb27-H1 binding to luminal CP43 and two Rubredoxins binding to stromal PsbE in higher plant Photosystem II as revealed by a structural mass spectrometry pipeline

Youngwoo Lee[1] and Haijun Liu[1]

[1]*Department of Biology, Saint Louis University, St. Louis, MO 63103, USA. ylee015@slu.edu*

Cross-linking mass spectrometry (XL-MS) is a structural proteomics technique used to study protein–protein interactions and protein conformations at residue-level resolution. It has become a powerful complement to cryo-EM, X-ray crystallography, and NMR, particularly for probing dynamic or heterogeneous complexes. Here, we present a pipeline of locating protein subunits in large protein complexes by integrating isotopically encoded cross-linking, identification, evaluation, structural prediction, modeling, and structure validation. Using Photosystem II (PSII) as a model, the structural location of TLP18.3, Psb27, and two Rubredoxins in Photosystem II on the luminal side and stromal sides respectively in higher plants were established. Protein structure prediction, including AlphaFold 3, was performed if structures were not available. Structural modeling was performed using HADDOCK and ClusPro and importantly justified by using the chemical restraint, i.e., the intrinsic feature of the cross-linker arm span (11 Å). The resulting models of TLP18.3-Psb27-PSII and Rubredoxin-PSII provide a solid foundation for understanding their molecular functions during PSII dynamic life cycle and steady-state photoprotection in higher plants.

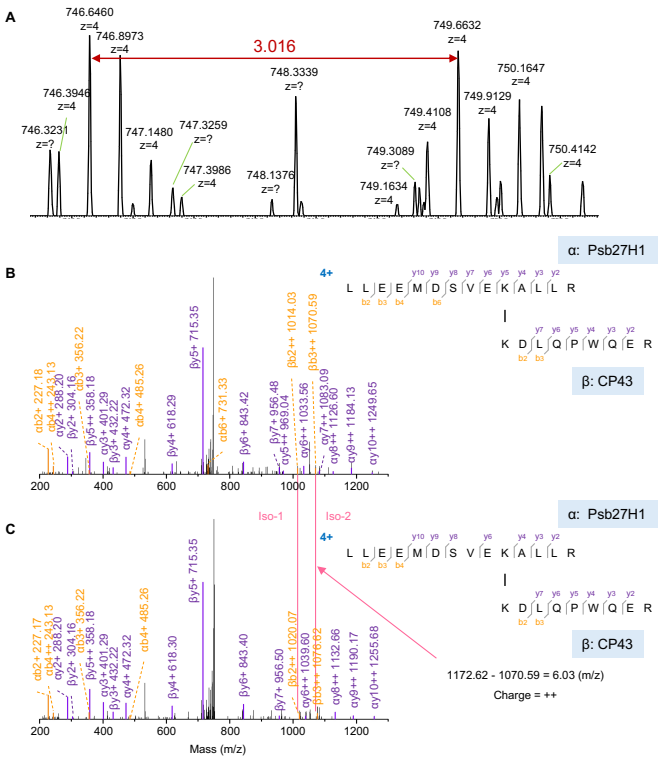

Figure. 1. Cross-links between Psb27 and CP43.

a, Precursor ion (MS1) spectra of cross-linked Psb27 and CP43 peptides showing the light and heavy cross-linked species (BS_3-h_{12}/d_{12}). The characteristic isotopic fingerprint appears as a peak doublet of similar intensity, separated by m/z 3.016.

b, Product-ion spectra (MS2) of the cross-linked peptide with BS_3-h_{12}.

c, Product-ion spectra of the cross-linked peptide with BS_3-d_{12}.

Exemplary isotopic fingerprints are indicated by the product ion pair Iso-1 and Iso-2 in panel c, corresponding to two identical ions.