

# The structural cross-linking mass spectrometry justification of an AlphaFold2-generated PsbS structure of the elongated stromal loop region

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PsbS is a protein in Photosystem II (PSII) that is involved in non-photochemical quenching (NPQ), a process involved in shielding plants from excess light and preventing photoinhibition. Understanding the structure of PsbS more deeply could aid in understanding plants' mechanisms of adapting to excess light and varying environmental conditions which could enable new, yield-improving developments in agriculture. The structural location and function of PsbS in PSII, however, remain not fully understood, plausibly due to intrinsically disordered regions (IDRs) in the N-terminal domain and stromal loop that prove difficult to characterize via X-ray crystallography or cryo-EM. This study used cross-linking mass spectrometry (XL-MS), a methodology that allows for protein residues that lie in proximity to be chemically linked and subject to interrogation via mass spectrometry. Isotopically encoded intrachain cross-links of PsbS were used as spatial constraints to critically justify AlphaFold2. This research highlights the combinatorial use of structural mass spectrometry and *in silico* protein structure prediction to quickly produce a whole proteome protein structure of an organism.