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Crosslinking mass spectrometry unveils novel interactions and structural distinctions in the model green alga *Chlamydomonas reinhardtii*

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Affiliations

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Supplemental Information

Supplementary Information 1. The Proteome Discoverer processing (left) and consensus (right) workflows and corresponding settings. If parameters are not listed, the default settings were used.

Supplemental Figures

Figure S1. Representative CSM showing the annotated MS2 spectrum of the interlink between K157 of cytosolic 80S ribosomal protein S27a (Cre06.g273600.t1.2) and K65 of cytosolic 80S ribosomal protein S15 (Cre08.g360900.t1.2). Reporter ions are labeled and shown here in blue and red.

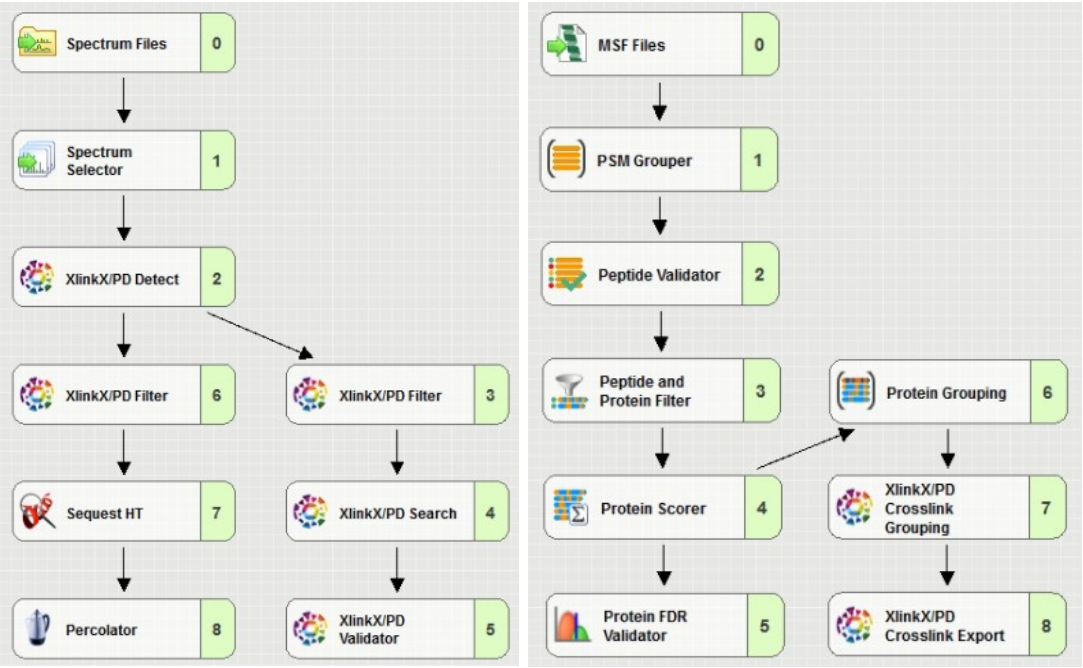
Figure S2. Predicted protein structures of Elongation Factor 3 and ABC transporter (Cre04.g222700.t1.2) using I-TASSER incorporating all detected intralinks. The top 5 structures with mapped intralinks are shown and all crosslinks had measured distances within the maximum restraint for DSSO (in green).

Figure S3. Predicted protein structure of Mg-chelatase (Cre06.g306300.t1.2) using I-TASSER incorporating all detected intralinks. The top structure with mapped intralinks is shown and all crosslinks had measured distances within the maximum restraint for DSSO (in green).

Figure S4. Preliminary complex modeling between Mg-chelatase (Cre06.g306300.t1.2, teal) and MCAP (Cre11.g477733.t1.2, orange) using I-TASSER and HADDOCK incorporating all detected interlinks. The top structures from each cluster with mapped interlinks are shown and ordered by the number of interlinks that had measured distances within the maximum restraint for DSSO (in green).

Figure S5. Refined complex modeling between Mg-chelatase (Cre06.g306300.t1.2, teal) and MCAP (Cre11.g477733.t1.2, orange) using I-TASSER and HADDOCK incorporating compatible interlinks from the first structure in Figure S4. The top structures from each cluster with mapped interlinks are shown and ordered by the number of interlinks that had measured distances within the maximum restraint for DSSO (in green).

Supplementary Information 1. The Proteome Discoverer processing (left) and consensus (right) workflows and corresponding settings. If parameters are not listed, the default settings were used.



Processing Workflow Settings

Spectrum Selector (1)

Precursor Selection	Use MS(n-1) with Parent Precursors
Min. Precursor Mass	350 Da
Max. Precursor Mass	10000 Da
Unrecognized Activation Type Replacements	HCD

XlinkX/PD Detect (2)

Acquisition strategy	MS2
Crosslink Modification	DSSO / +158.004 Da (K)
Enable protein N-terminus	True

XlinkX/PD Filter (3)

Select	Crosslinks

XlinkX/PD Search (4)

Enzyme Name	Trypsin (Full)

Maximum Missed Cleavages	2
Precursor Mass Tolerance	15 ppm
Fragment Mass Tolerance	0.02 Da
Static Modification	Carbamidomethyl / +57.021 Da (C)
Dynamic Modification	Oxidation / +15.995 Da (M)
Dynamic Protein N-term Modification	Acetyl / +42.011 Da (N-Terminus)

XlinkX/PD Validator (5)

FDR threshold	0.01

XlinkX/PD Filter (6)

Select	Peptides

Sequest HT (7)

Enzyme Name	Trypsin (Full)
Maximum Missed Cleavages	2
Precursor Mass Tolerance	15 ppm
Fragment Mass Tolerance	0.02 Da
Weight of a Ions	1
Weight of b Ions	1
Weight of y Ions	1
Max. Equal Modifications Per Peptide	3
Static Modification	Carbamidomethyl / +57.021 Da (C)
Dynamic Modification	Oxidation / +15.995 Da (M) DSSO Tris / +279.078 Da (K) DSSO Hydrolyzed / +176.014 Da (K)
Dynamic Protein N-term Modification	Acetyl / +42.011 Da (N-Terminus)

Percolator (8)

Target/Decoy Selection	Concatenated
Target FDR (Strict)	0.01
Target FDR (Relaxed)	0.05

Consensus Workflow Settings

MSF Files (0)

Spectra to Store	Identified
Reported FASTA Title Lines	Best match

PSM Grouper (1)

Site Probability Threshold	75

Peptide Validator (2)

Target FDR (Strict) for PSMs	0.01
Target FDR (Relaxed) for PSMs	0.05
Target FDR (Strict) for Peptides	0.01
Target FDR (Relaxed) for Peptides	0.05

Peptide and Protein Filter (3)

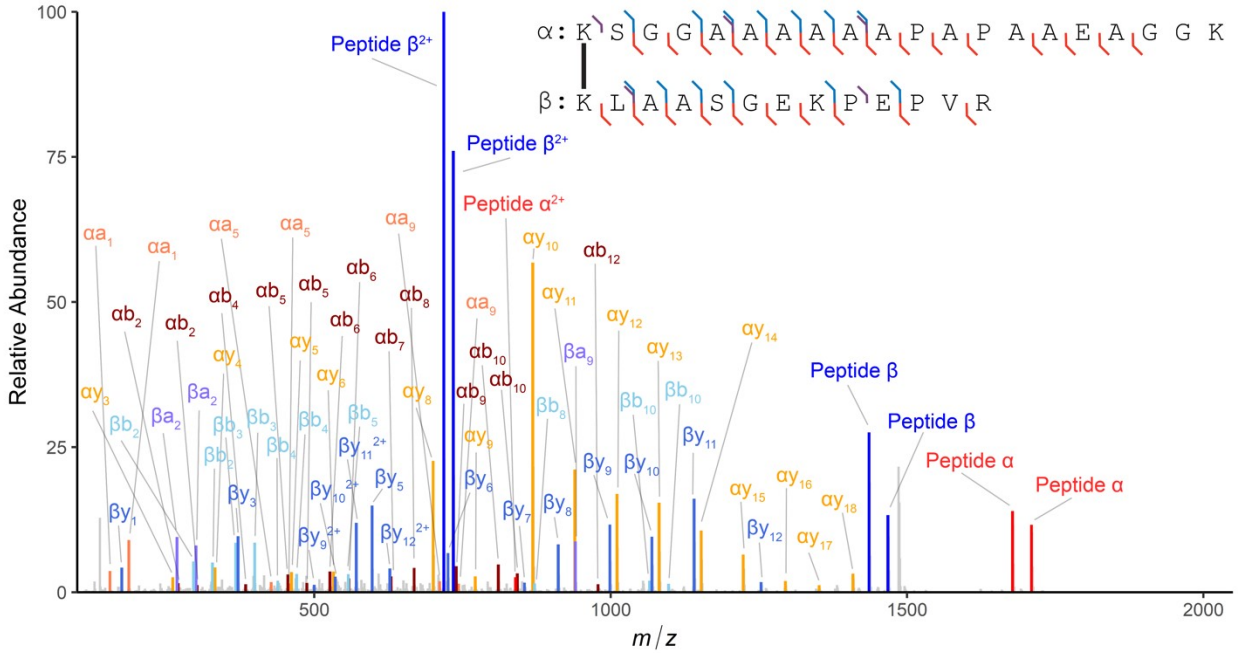
Peptide Confidence At Least	High
Minimum Number of Peptide Sequences	1

Protein FDR Validator (5)

Target FDR (Strict)	0.01
Target FDR (Relaxed)	0.05

Protein Grouping (6)

Apply strict parsimony principle	True



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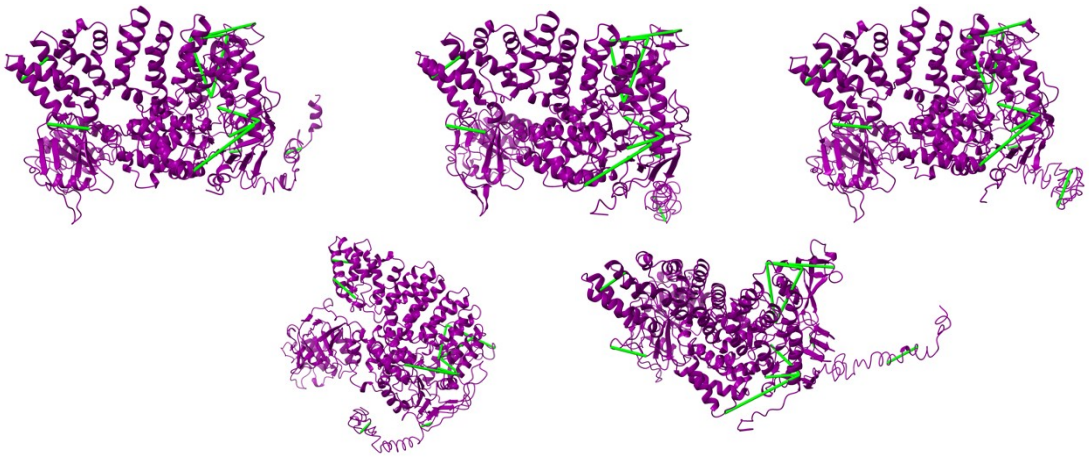

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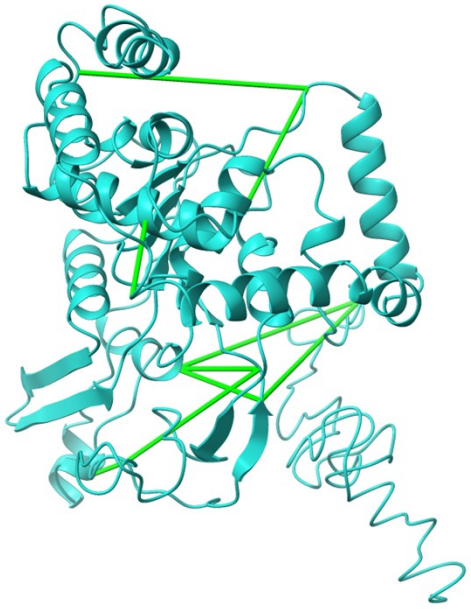

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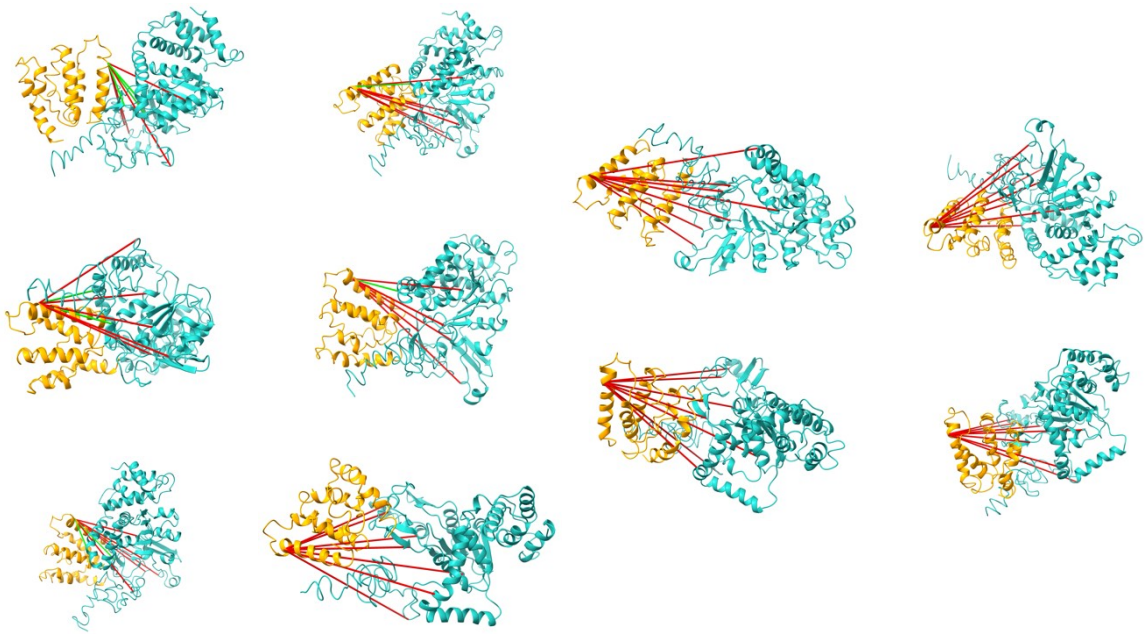

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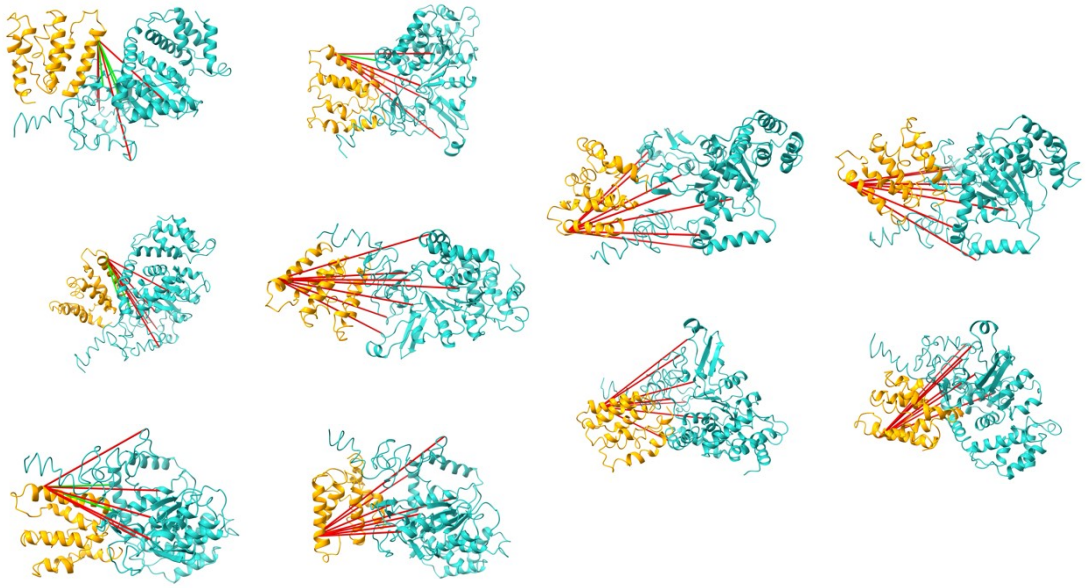

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