Proteomics

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Recently developed Open Tools and Resources for Arabidopsis Researchers

Members of the subcommittee have been working on an updated portal for the visualization of Arabidopsis MS data ever since the closure of the MASCP Gator. This new portal has now been finalized and is available through the PeptideAtlas: http://www.peptideatlas.org/builds/arabidopsis/

Recent or Future activities of Subcommittee members

The last few years has witnessed several new surveys of Arabidopsis proteins by mass spectrometry. These approaches (deep proteomics) occurred due to the significant improvements in instrumentation, mainly sensitivity, that have occurred over the past 5 years. These data have been re-analyzed and combined into a PeptideAtlas portal that will enable the community to easily visualize peptide coverage on a given Arabidopsis protein. This new portal will enable additional information to be captured (e.g. PTMs, quantitation) about Arabidopsis proteins and is likely to be better supported into the future due to it being hosted by the institute for Systems Biology along with other model system proteomes (e.g. human, Drosophila, C.elegans, etc).

Selected Publications

• Smith S, Zhu S, Joos L, Roberts I, Nikonorova N, Vu LD, Stes E, Cho H, Larrieu A, Xuan W, Goodall B, van de Cotte B, Waite JM, Rigal A, Ramans Harborough S, Persiau G, Vanneste S, Kirschner GK, Vandermarliere E, Martens L, Stahl Y, Audenaert D, Friml J, Felix G, Simon R, Bennett MJ, Bishopp A, De Jaeger G, Ljung K, Kepinski S, Robert S, Nemhauser J, Hwang I, Gevaert K, Beeckman T, De Smet I (2020) The CEP5 Peptide Promotes Abiotic Stress Tolerance, As Revealed by Quantitative Proteomics, and Attenuates the AUX/IAA Equilibrium in Arabidopsis. Mol Cell Proteomics 19: 1248-1262

Showcases the power of quantitative proteomics to identify pathways, in this instance a novel peptide-dependent control mechanism that tunes auxin signaling.

• Bassal M, Abukhalaf M, Majovsky P, Thieme D, Herr T, Ayash M, Tabassum N, Al Shweiki MR, Proksch C, Hmedat A, Ziegler J, Lee J, Neumann S, Hoehenwarter W (2020) Reshaping of the *Arabidopsis thaliana* Proteome Landscape and Co-regulation of Proteins in Development and Immunity. Mol Plant 13: 1709-1732

A further addition to the recent deep proteome analyses of Arabidopsis, this study defines the absolutely quantification of 16000 proteins throughout the plant lifecycle.

• Heinemann B, Kunzler P, Eubel H, Braun HP, Hildebrandt TM (2021) Estimating the number of protein molecules in a plant cell: protein and amino acid homeostasis during drought. Plant Physiol 185: 385-404

A unique look at the balance between protein and amino acid pools in drought stressed Arabidopsis.

• Fu L, Liu Y, Qin G, Wu P, Zi H, Xu Z, Zhao X, Wang Y, Li Y, Yang S, Peng C, Wong CCL, Yoo SD, Zuo Z, Liu R, Cho YH, Xiong Y (2021) The TOR-EIN2 axis mediates nuclear signalling to modulate plant growth. Nature 591: 288-292

Good demonstration of how protein mass spectrometry (phosphoproteomics) can be used to uncover new functions, in this instance phosphorylation of EIN2
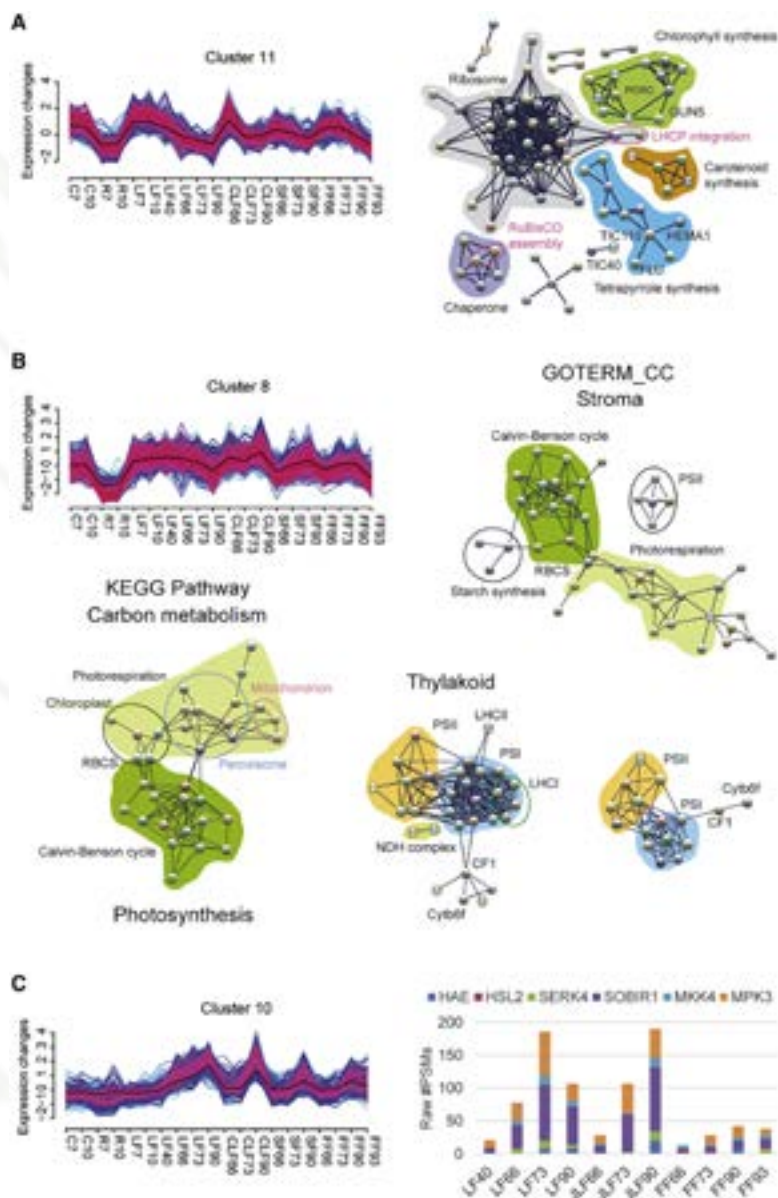
• Tang Y, Huang A, Gu Y (2020) Global profiling of plant nuclear membrane proteome in Arabidopsis. Nat Plants 6: 838-847

Application of emerging technique of proximity-labelling technology coupled to quantitative mass spectrometry to examine the nuclear envelope proteins in Arabidopsis.

Planning for Fourth Decadal Roadmap

Overall: improvements in our ability to better understand protein dynamics within the cell and the plant during development and stress conditions.

• Single cell proteomics

• Dynamics and function of PTMs (stoichiometry, competition)

• Protein turnover

• Whole proteome quantification (simple as RNAseq)

• Improved targeted analysis (simple as qPCR)

• Protein interactions (complexes vs transient interactions)

• Subcellular proteomics. A complete map and dynamics

• Integration with other technologies for predictive modelling of plant development and response

Proteome Remodeling in the Establishment and Maintenance of Photosynthesis, Degradation of Photosynthetic Apparatus, and Leaf Senescence.
(A) FCM cluster 11. Right panel: physical/functional protein interaction networks generated with the STRING database using all proteins assigned to the GOTERM category Chloroplast (Supplemental Table 12) as the input set. Various biochemical pathways involved in pigment- and photosynthesis-related protein synthesis and chloroplast biogenesis are color coded and indicated.
(B) FCM cluster 8. Right panel: physical/functional protein interaction networks generated with the STRING database using all proteins assigned to the GOTERM categories Stroma, Thylakoid, Carbon metabolism (C Metabolism), and Photosynthesis as input sets (Supplemental Table 13). Light-independent reactions are highlighted in green (Calvin-Benson cycle, dark; photorespiration, light) and light-dependent reactions in orange (photosystem II [PSII]) and blue (photosystem I [PSI]).

Systems and Synthetic Biology

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with support from
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(i) Belcher, M.S., Vuu, K.M., Zhou, A. *et al*. Design of orthogonal regulatory systems for modulating gene expression in plants. Nat Chem Biol 16, 857–865 (2020).

A library of synthetic transcriptional regulators (activators, repressors and promoters) that modulate expression strength in planta.

(ii) Brooks MD, Juang C-L, Katari MS, Alvarez JM, Pasquino H-J, Huang J, Shanks C, Cirrone J, Coruzzi GM. ConnecTF: A platform to integrate transcription factor–gene interactions and validate regulatory networks. Plant Physiology. 185(1). (2021)
A species-independent, web-based platform that integrates genome-wide stdies of TF-target binding, TF-target regulation and other TF-centric omic datasedt and uses these to build and refine validated or inferred gene regulatory networks.

(iii) Cai YM, Carrasco Lopez JA, Patron NJ. Phytobricks: Manual and Automated Assembly of Constructs for Engineering Plants. Methods in Molecular Biology. 2205:179-199.

Phytobricks are standardized DNA parts for plants that can be assembled hierarchically into transcriptional units and multigene constructs. This protocol describes Phytobrick design and construction and their assembly in manual and nanoscale automated one-step reactions as well as high-throughput sequence verification of assembled plasmids.

(iv) Lehmann S., Dominguez-Ferreras A., Huang W., Denby K., Ntoukakis V., Schäfer P. Novel markers for high-throughput protoplast-based analyses of phytohormone signaling.Plos One 15: e0234154 (2020)
The authors generated and validated 18 promoter::luciferase and suggest an experimental setup for high-throughput analysis. They recommended novel markers for the analysis of auxin, abscisic acid, cytokinin, salicylic acid and jasmonic acid responses.

(v) Cirrone J, Brooks MD, Bonneau R, Coruzzi GM, Shasha DE. OutPredict: multiple datasets can improve prediction of expression and inference of causality. Scientific Reports. 10(1):6804. (2020)

Prediction of causal gene regulatory relationships based on time series data as well as known network edges and steady-state data. The method across different species has improved predictive accuracy over other state-of-the-art methods.