Metabolic Response to Altered Light Conditions in Genetically Modified *Chlamydomonas* Followed by LAESI Mass Spectrometry with Ion Mobility Separation

Sylwia A. Stopka,[1] Bindesh Shrestha,[1] Éric Maréchal,[2] Denis Falconet[2] and Akos Vertes[1]

[1]Department of Chemistry, W. M. Keck Institute for Proteomics Technology and Applications, The George Washington University, Washington, DC 20052
[2]Laboratoire de Physiologie Cellulaire Végétale, UMR 5168, CEA-CNRS-INRA-Université Grenoble Alpes, Grenoble, France

The microalga *Chlamydomonas reinhardtii* has been extensively studied for its relevance in biofuel production. This fully sequenced model organism has applications in focused areas such as photosynthesis, metabolism, cell cycle, motility, and genetics.[1] To improve the understanding of lipid biosynthesis and its implications for using *Chlamydomonas* as a biofuel source, factors such as environmental conditions and selection of strains with particular genotypes need to be further investigated. Depending on nutrient availability and environmental conditions C. reinhardtii has the ability to store energy as starch or as neutral lipids, e.g., triacylglycerols (TAG). Here we demonstrate the use of a robust ambient analytical tool, laser ablation electrospray ionization (LAESI) mass spectrometry (MS) with ion mobility separation (IMS), to better understand metabolic changes and adaptations at the biochemical pathway level under varied stress conditions.

C. reinhardtii mutants with an impaired starch pathway were investigated for the production of neutral storage lipids under altered light conditions. Starch synthesis is inhibited by disrupting the central enzyme of ADP-glucose pyrophosphorylase (AGPase-SS) that is responsible for the formation of glucosyl nucleotides from glucose-1-phosphate with ATP in the starch pathway. This enzyme is activated by 3-phosphoglyceric acid (3-PGA). The *Sta*1 mutant has a disrupted large catalytic subunit of AGPase-SS, and as a consequence it exhibits a reduced activation by 3-PGA. This allows the mutant to retain less than ~10% of its normal starch production.[2] The *Sta*6 mutant has a disrupted small catalytic subunit of AGPase-SS and as a result only retains ~1% of the normal starch levels.[3]

Conventional lipid analysis of microalgae consists of extensive extraction protocols and/or derivatization, resulting in lengthy runs and the loss of some structural information. To reduce the complexity of the sample, a separation step that distinguishes the different classes of compounds and isobaric species is required. LAESI-MS is an ambient ionization technique that has been employed to detect metabolites, lipids, and peptides from diverse biological samples. This technique utilizes the natural water content of cells and tissues to facilitate laser energy deposition by the strong absorption of water at 2.94 μm wavelength and to produce an ablation plume. The plume is then ionized by an electrospray and sampled by a high-performance Q-TOF mass spectrometer (Synapt G2 S, Waters Co.). A typical LAESI mass spectrum of *Chlamydomonas* contains approximately ~250 detected ions but it does not differentiate between structural isomers and isobaric species. Combination of LAESI-MS with IMS has been shown to produce a 3 fold increase in molecular coverage.[4] The resulting enhanced data includes *m/z* ratios, drift times (DT) related to the collision cross section of the ion, and peak intensities. To reduce spectral interferences from the medium and to prevent osmotic shock the cells are deposited in a silica membrane spin tube and the supernatant is separated from the cells by centrifuging at 2,000×g for 1 min. The laser pulses are directly coupled into the remaining pellet of microalgae on the filter.

Initially the wild type (WT) *C. reinhardtii* was studied under low and high light conditions, with photosynthetically active radiation (PAR) 0 and 150 $\mu mol \cdot m^{-2} sec^{-1}$, respectively. After a 72 h period, the cells were harvested and LAESI-IMS-MS was performed. In the lipid region of the spectra, normalized for the most abundant diacylglyceryl-N,N,N-trimethylhomoserine (DGTS) ion, DGTS(34:3), enhanced production of monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols (DGDG) and TAG lipids was observed under high light conditions.

The *Sta*1 and *Sta*6 mutants were investigated for lipid production in altered light conditions ranging from 0 to 200 $\mu mol \cdot m^{-2} sec^{-1}$ over a 72 h time period. When compared to the WT cells, significant levels of TAG and DGDG were observed in both mutants throughout the 72 h time period. Using LAESI-MS without IMS ~10 TAG lipid species were detected. The introduction of IMS improved the molecular coverage of TAG lipids to ~50 species in the DT range of 189 to 204 ms (see Figure 1a).

When compared to WT, the *Sta*1 mutant showed stronger ion signal for TAG lipids within $800 < m/z < 880$ at 72 h under high light condition. Conversely, the *Sta*6 mutant presented the strongest ion signal for TAG lipids at 72 h in low light illumination. Under opposite lighting conditions, the two *Sta* mutants exhibited similar TAG lipid profiles.

To reduce spectral interferences and differentiate close to isobaric species, for example TAG(54:7) and the ^{13}C peak of chlorophyll *a*, both at nominal *m/z* 894, two DT ranges were inspected. In the LAESI-IMS-MS spectrum, the DT range integrated between 201 and 207 ms revealed the TAG(54:7) species at *m/z* 894.7571, whereas the DT range integrated between 181 and 188 ms showed the ^{13}C isotope peak of chlorophyll *a* at *m/z* 894.5512 (see Figure 1b).

These results demonstrate our ability to observe numerous changes in lipid levels in WT and genetically modified microalgae populations affected by light exposure. We found that impaired starch pathways *C. reinhardtii* result in energy storage redirected to neutral lipids. These capabilities are expected to accelerate the research on the utility of genetically altered microalgae strains for biofuel production.

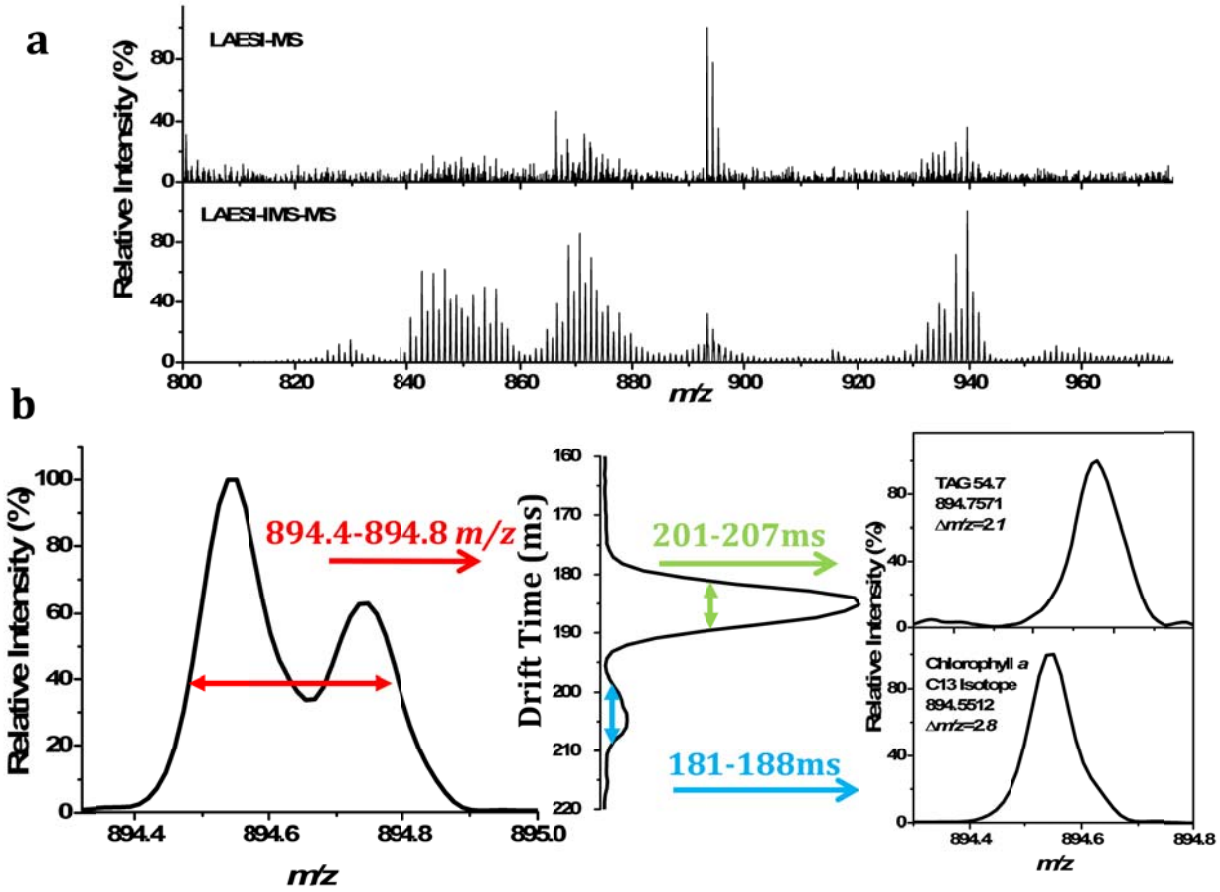

Figure 1. (a) Molecular coverage of TAG lipid species with LAESI-MS (top) and LAESI-IMS-MS in the DT range of 189 to 204 ms (bottom). **(b)** An *m/z* range around the close to isobaric ions at *m/z* 894 (left) in the LAESI-MS spectrum was selected to reveal the corresponding DT distributions (middle). Mass spectra for the ions with distinct DT revealed two separate ionic species, chlorophyll *a* and TAG(54:7) (right).

1. E. H. Harris, *Annual Review of Plant Physiology and Plant Molecular Biology*, 2001, **52**, 363-406.
2. C. Zabawinski, N. Van den Koornhuyse, C. D'Hulst, R. Schlichting, C. Giersch, B. Delrue, J. M. Lacroix, J. Preiss and S. Ball, *Journal of Bacteriology*, 2001, **183**, 1069-1077.
3. V. H. Work, R. Radakovits, R. E. Jinkerson, J. E. Meuser, L. G. Elliott, D. J. Vinyard, L. M. L. Laurens, G. C. Dismukes and M. C. Posewitz, *Eukaryotic Cell*, 2010, **9**, 1251-1261.
4. B. Shrestha and A. Vertes, *Analytical Chemistry*, 2014, **86**, 4308-4315.