Supplemental Information for

Population Screening of Chlamydomonas reinhardtii with Single-Cell Resolution Using a High-throughput Micro Scale Sample Preparation for MALDI Mass-Spectrometry.

Authors:

Jasmin Krismer[1], Jens Sobek, Robert F. Steinhoff[1], Stephan R. Fagerer[1], Pabst Martin[1], Renato Zenobi[1]

Affilitations

[1]Department of Chemistry and Applied Biosciences, Laboratory of organic Chemistry, ETH Zurich, Switzerland

[2]Functional Genomics Center Zürich, ETH Zurich and University of Zurich, Switzerland

Table S 1 Sample preparation protocol for high-throughput single-cell measurements

Protocol for the preparation of microbial cells for a single cell experiment using MALDI microarray plates.

1) The empty microarray slide is scanned to check for auto-fluorescent dust.
2) 10 drops (4-5 nanoliter) of MALDI-matrix solution (for DHB 10mg/ml in 80 % acetone 30 % water) are spotted into each well of the array.
3) The cells are washed with deionized water prior to the spotting. For this purpose cells are centrifuged three times (2000 x g for 5 min in the case of *Chlamydomonas reinhardtii*) and re-suspended in water (HPLC grade) to remove salts and lysate that might suppress or bias signals measured in single-cell MALDI-MS.
4) A dilution series of cells is spotted to determine the number of drops resulting in the maximum number of wells, containing single cells. A concentration of around 10^6 cells per ml should result in an average of 0.5 cells per drop(≈0.5 nl). By testing the results for 1,2,3,5 or 10 drops one can account for effects like clustering or cell divisions. The Fluorescence scan reveals the number of drops for maximal single cell yield.
5) Cells are then spotted onto the whole array and then subjected to a fluorescence scan.
6) Cells are then lysed and their metabolism quenched by submerging the slide in liquid nitrogen. The cell walls are cracked by the immediate crystallization of water inside the cells. The slide is reconstituted to room temperature in a desiccator to avoid condensation on the slide that could reposition cells or lead to cross-contamination between wells. To check for cell displacements, the slide can be subjected to another fluorescence scan.
7) Then MALDI matrix is applied onto the cracked cells to extract analytes from the cells and co-crystallize them with the MALDI-matrix. For *Chlamydomonas reinhardtii* we found that 50 drops(20-25 nanoliter) applied in 5 consecutive spotting runs of 10 drops worked best. Applying 10 drops of matrix consecutively means that analytes are extracted in five consecutive extractions after each of which matrix crystallizes with the extracted analytes, which makes this step more robust and reproducible. To monitor the extraction process the slide is subjected to another fluorescence scan.
8) Cells are then put into plastic tubes and stored under a nitrogen atmosphere and stored at -80° C until measured with MALDI-MS.

Table S 2 Compounds detected in a single *Chlamydomonas reinhardtii* cell using MALDI-FT-ICR-MS

	Compound name	Formula	Measured m/z	Theoretical m/z	δ (ppm)
Thylakoid lipids	MGDG (34:7) + Na^+	C43H68O10	767.47026	767.47047	0.3
	MGDG (34:7) + K^+	C43H68O10	783.44431	783.44441	0.1
	DGDG(34:6) + Na^+	C49H80O15	931.53888	931.53894	0.1
	DGDG(34:5) + Na^+	C49H82O15	933.55462	933.55459	0.0
	DGDG(34:3) + Na^+	C49H86O15	937.58611	937.58589	0.2
	DGDG(34:2) + Na^+	C49H88O15	939.60178	939.60154	0.2
Membrane Lipids	DGTS (32:4)	C42H73NO7	704.54577	704.54598	0.3
	DGTS (32:3)	C42H75NO7	706.56126	706.56163	0.5
	DGTS (32:2)	C42H77NO7	708.57761	708.57728	0.5
	DGTS (32:1)	C42H79NO7	710.59311	710.59293	0.3
	DGTS (34:5)	C44H75NO7	730.56125	730.56163	0.5
	DGTS (34:4)	C44H77NO7	732.57698	732.57728	0.4
	DGTS (34:3)	C44H79NO7	734.59267	734.59293	0.4
	DGTS (34:2)	C44H81NO7	736.60863	736.60858	0.1
	DGTS (36:5)	C46H79NO7	758.59257	758.59293	0.5
	DGTS (36:4)	C46H81NO7	760.60822	760.60858	0.5
	DGTS (36:3)	C46H81NO7	762.62399	762.62423	0.3
Pigments	Chlorophyll a (-Mg^{2+}+3H^+)	C55H74N4O5	871.57313	871.5732	0.1
	Chlorophyll b (-Mg^{2+}+3H^+)	C55H72N4O6	885.55288	885.55246	0.5
	Cartenoid (Canthaxanthin)	C10H52O2	565.40431	565.50501	0.5
	Cartenoid (Fritschellaxanthin)	C40H54O3	583.41505	583.41457	0.8
	Carotenoid (Violaxanthin/Neoxanthin)	C40H56O4	601.42546	601.42514	0.5

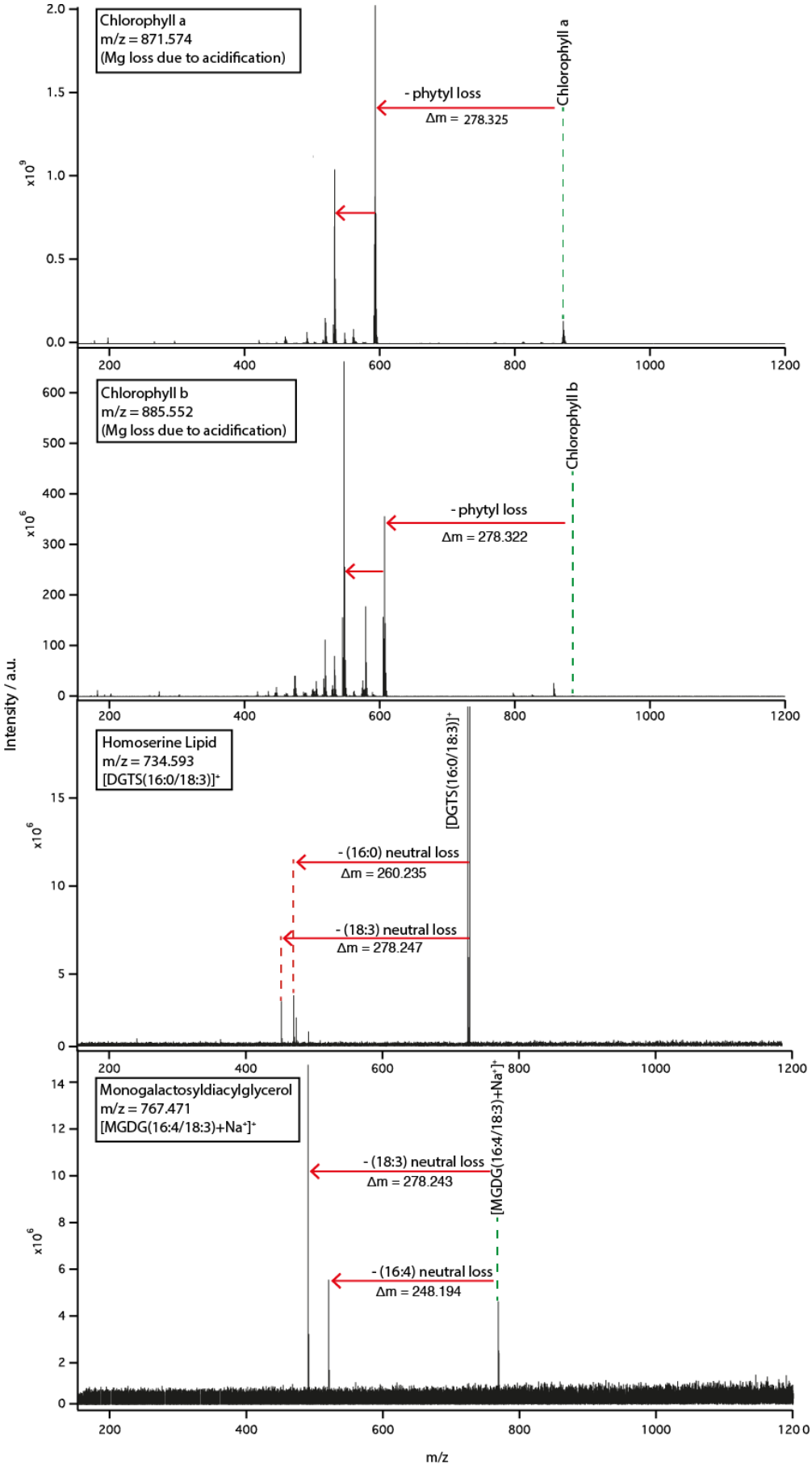

Figure S 1 MS/MS spectra of the different lipid classes detected in Chlamydomonas reinhardtii.

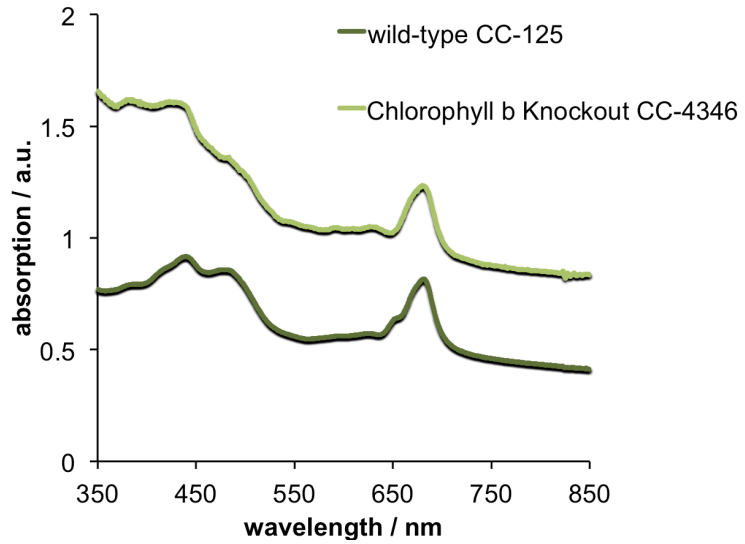

Figure S 2 UV-VIS spectra of Chlamydomonas reinhardtii cell cultures.

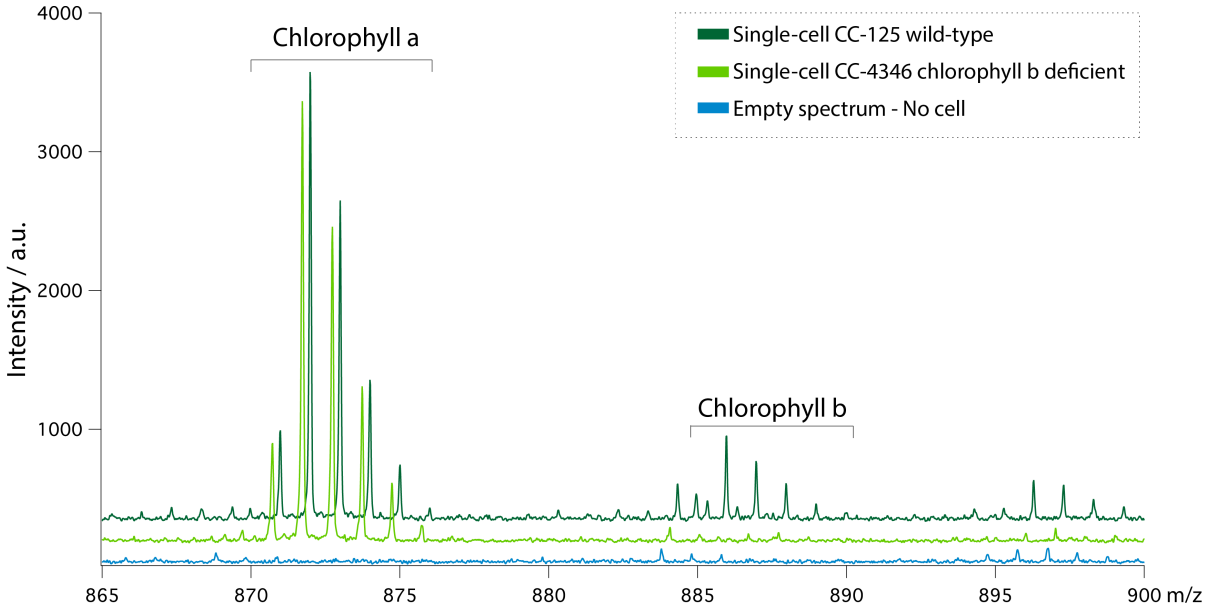

Figure S 3 Chlorophylls detected from single cells. The wild-type strain shows both chlorophyll a and chlorophyll b while the chlorophyllide a oxidase mutant shows only chlorophyll a and no chlorophyll b. Thus the two strains can be differentiated based on the presence or absence of chlorophyll b.

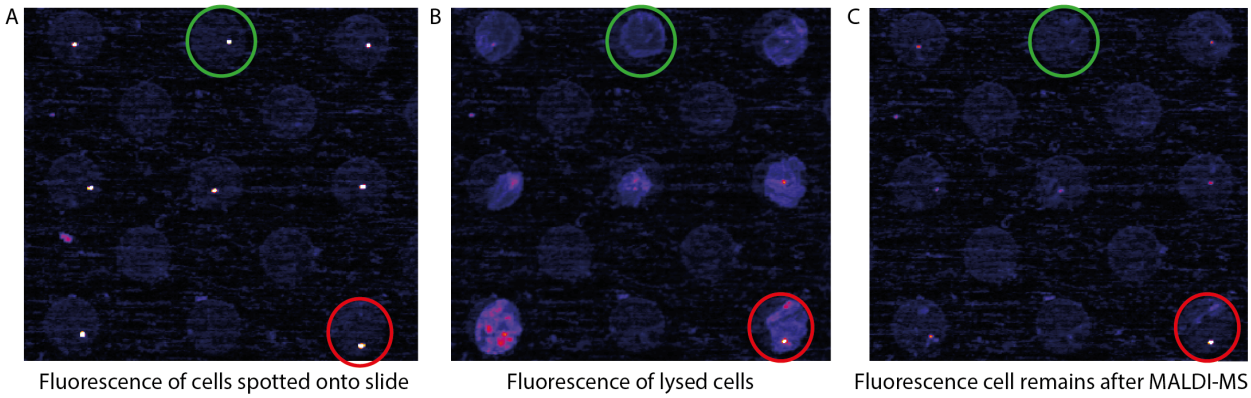

Figure S 4 Effect of incomplete cell lysis on the depletion of chlorophyll fluorescence by the MALDI ablation process. A Single cells are placed into microarray spots. B Chlorophyll that stays inside the cell is not co-crystallized with matrix and therefore ionized much less efficiently. Efficient lysis is therefore a prerequisite for reproducible and good quality mass spectra. C Chlorophyll that is not extracted stays inside the cells despite the laser ablation.