# Biological material

Axenic *Tisochrysis lutea* CCAP 927/14 was cultured in duplicate in chemostat in cobalamin limitation and in nitrogen limitation. Read (Nef et al., 2019) for culture informations. For each one of the 4 cultures, 3 samples were done at steady state and on sample one hour after discret addition of the limiting nutrient.

Cell lysis was performed by suspending 350 106 cells in 500 µL Valot buffer (Valot, 2005) and 500 µL phenol pH 8 with protease inhibitor (complete tablets, Roche Diagnostics, Mannheim, Germany) and sonicating 2 min in ice. After centrifugation (10,000 g, 10 min, 4°C), proteins of supernatant were precipitated with 6 volumes acetone overnight at -20°C, then washed twice with hydrochloride guanidine-ethanol (0.3 M) and one with ethanol 70 %. Finally, pellets were dried and suspended in Tris-HCl 0.5 M pH 7.5 buffer with 1 % SDS. Proteins were quantified using a Qubit 3 fluorometer (Invitrogen, Thermo Fisher Scientific) following the manufacturer’s instruction and 20 μg proteins were loaded in SDS-PAGE 10 % acrylamide gel. Each lane was cut into three bands of identical size and washed for 15 min with an acetonitrile/100 mM ammonium bicarbonate mixture (1:1). In-gel digestion was performed in 50 mM ammonium bicarbonate pH 8.0 and the quantity of modified trypsin (Promega, sequencing grade) was 0.1 μg per sample. Digestion was achieved for 6 h at 37°C and supernatant was kept. Peptides were extracted by 5 % formic acid in water/acetonitrile (v/v). Supernatant and extracted tryptic peptides were dried and suspended in 20 µL of 0.1 % (v/v) formic acid and 2 % (v/v) acetonitrile.

# Liquid Chromatography – Mass Spectrometry

Mass spectrometry was performed on the PAPPSO (Plateforme d’Analyse Protéomique de Paris Sud-Ouest) platform (MICALIS, INRA, Jouy-en-Josas, France; http://pappso.inra.fr/). An Orbitrap Fusion™ Lumos™ Tribrid™ (Thermo Fisher Scientific) coupled to an UltiMate™ 3000 RSLCnano System (Thermo Fisher Scientific) was used. A 4 μL sample was loaded at 20 μL/min on a precolumn (µ-Precolumn, 300 µm i.d x 5 mm, C18 PepMap100, 5 µm, 100 Å, Thermo Fisher) and washed with loading buffer. After 3 min, the precolumn cartridge was connected to the separating column (Acclaim PepMap®, 75 μm x 500 mm, C18, 3 μm, 100 Å, Thermo Fisher). Buffer A consisted of 0.1 % formic acid in 2 % acetonitrile and buffer B of 0.1 % formic acid in 80 % acetonitrile. The peptide separation analysis was achieved at 300 nL/min with a linear gradient from 1 to 28 % buffer B for 105 min and 28 % to 40 % for 10 min. One run took 147 minutes including the regeneration step at 98 % buffer B. Ionization (1.6 kV ionization potential) and capillary transfer (270°C) were performed with a liquid junction and a capillary probe (SilicaTip™ Emitter, 10 μm, New Objective). Peptide ions were analyzed using Xcalibur 3.1.66.10 in DDA mode with cycle time of 3 seconds. In HCDOT mode the machine settings were as follows: 1) full MS scan in Orbitrap (scan range [m/z] = 400–1500) with a resolution of 120 000 (AGC target = 5.0 x 105, max. injection time = 15 ms, data type = profile). The quadrupole isolation mode was 1.2. Analyzed charge states were set to 2-5, the dynamic exclusion to 80 s and the intensity threshold was fixed at 5.104 and 2) MS/MS HCD (30 % collision energy) in Orbitrap (AGC target = 5.0 x 104, max. injection time = 54 ms).

# Protein identification

The *T. lutea* strain database from Garnier et al. (2016) (version 2018, 17373 entries) was searched by the X!TandemPipeline (open source software developed by PAPPSO, version 0.2.21, <http://pappso.inra.fr/bioinfo/xtandempipeline>). Protein identification was run with a precursor mass tolerance of 5 ppm and a fragment mass tolerance of 10 ppm. Enzymatic cleavage rules were set to trypsin digestion (“after Arg and Lys, unless Pro follows directly after”) and no semi-enzymatic cleavage rules were allowed. The fixed modification was set to cysteine carbamidomethylation and methionine oxidation was considered as a potential modification. Common contaminants such as human keratins were added to the databank. The identified proteins were filtered as follows: 1) peptide *e*-value < 0.01 with a minimum of 2 peptides per protein and 2) protein *e*-value < 10-4. A reversed sequence database was used to calculate False Discovery Rates (FDR). A 0.05 % FDR was achieved at peptide level and 0.00 % at protein level.

# Data treatment and statistical analyses

Alignment of the different lanes was performed using MassChroQ (open source software developed by PAPPSO, <http://pappso.inra.fr/bioinfo/masschroq>). Peptide quantities were analyzed by eXtracted Ion Current (XIC) using MassChroQ “Black Caiman” version 0.3.7 (Valot et al., 2011). The range for peak detection was set to 10 ppm with a detection threshold ranging from 30000 to 50000. Data were finally analysed with MassChroQR package for R 3.5.1. MassChroQ data gathering quantification information were attached to their corresponding metadata using a template on Notepad++. Peptides having a standard deviation relative to the retention time of 20 s or more and peptides having a peak width greater than 80 s were eliminated. All peptide intensities were log10‑transformed for the following data treatments. The analysis using XIC required the alignment of retention times, intensity normalization and log-transformation of values. No imputation was made for peptide-mz intensity when a protein was completely absent from a sample. Protein abundances were calculated as the sum of peptide intensities, which allows for analysis of quantitative variations (Blein-Nicolas and Zivy, 2016). Shared peptides and proteins with less than three peptides were removed. Missing peptide intensities and protein abundances were assigned the lowest values recorded. The data set was normalized as follows: the peptide-mz intensities of a given injection were divided by the sum of all mz peptides intensities of the injection. The results obtained were multiplied by the average intensity computed over all injections. The function that allows peptide imputation uses an iterative robust model-based imputation from the R package VIM. As this function introduces a random error to the imputed values, the result might be different between the runs.

An ANOVA (analysis of variance) was performed to determine the significance of variation. The effect of the factor on protein accumulation was considered significant when *p* < 0.05 and when the fold of expression was superior to 1.8 or inferior to -1.8. Proteins were functionally annotated by protein domain identification using InterProScan and NCBI Conserved Domain Database (CDD) and using BlastP against Uniprot protein database (*e*-value threshold = 0.01). Expert curation was applied to validate results and processing final annotation.

**References**

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