2.1.Proteome Discoverer, which is a software trademark belonging to Thermo Fisher Scientific, provides access to annotation information from ProteinCenter, a web-based application used to retrieve biologically enriched data for individual proteins.Chemicals Water, methanol, acetonitrile (ACN), trifluoroacetic acid (TFA) and formic acid (FA), hexane, chloroform, Tris-HCl, ammonium bicarbonate (ABC), dithiothreitol (DDT), iodoacetamide (IAA), 2-cloroacetamide (CAA), sodium deoxycholate (SDC), trypsin, bovine serum albumin (BSA), C18 resin, nitric acid and Coomassie brilliant blue G-250 dye were purchased from Merck (Milan, Italy).Acquisitions in full scan mode were performed in the range of m/z 350-1600 with a resolution power of 120k using an automatic gain control (AGC) target of 3 x 106 and an injection time (IT) of 100 ms. Full-MS/ddMS2 acquisitions were conducted using normalized collision energy (NCE) fixed at 28 with a resolution of 15k, AGC of 1 x 105 , IT of 100 ms, isolation window of 1.2 m/z, minimum AGC of 1 x 103 and dynamic exclusion of 30 s. Instrumentation control is achieved using Xcalibur software 2.2 SP1.48 (Thermo Scientific).In solution digestion Protein digestion was performed starting from dried protein extracts obtained using Protocols I and II. The procedure began by adding 20 uL of 50 mM ABC and 2 uL of 100 mM dithiothreitol (DTT) to achieve a final DTT concentration of 10 mM. The mixture was incubated at 56 oC with shaking at 400 rpm for 30 min to reduce disulfide bonds.40 uL of 0.1 % TFA and 1 uL of resin C18 were ad alysis of extracted and purified protein digest samples was performed with an EASY-nLC(TM) 1200 nHPLC chromatograph (Thermo Fisher Scientific, USA) coupled with a Q-Exactive mass spectrometer (Thermo Scientific, USA) through an ESI interface.In the processing workflow, LC-MS conditions were set, including m/z tolerance (5 ppm for precursor mass, 0.05 Da for fragment mass), mass range (350-7000 Da for precursor mass), fragmentation type (higher-energy collisional dissociation, HCD), protease (trypsin), constant modification (carbamidomethylation of cysteine), and dynamic modification (oxidation of methionine).Bolt sample buffer, Nupage Bis-Tris gel 4-12 %, MES buffer, Instant Blue, prestained protein ladder, Bolt reducing agent and tris(2-carboxyethyl) phosphine (TCEP) were purchased from Thermo Fisher Scientific (Waltham, US).The supernatant was removed, and the protein pellet was dried under a nitrogen flow and stored at - 20 oC. Protocol III: Building upon a similar detergent combination described by Deb-Choudhury et al. (2016), this protocol was developed to enhance protein extraction efficiency through a synergistic interplay of key reagents (Deb-Choudhury et al., 2016).Proteins were then separated using precast NuPAGE(TM) Bis-Tris Plus gels (4-12 % gradient polyacrylamide) in MES SDS Running Buffer (Thermo Fisher Scientific), which provides optimal resolution for low to medium molecular weight proteins. Together, these reagents significantly boost protein yield and ensure a comprehensive extraction of proteins from diverse cellular compartments, providing a robust foundation for downstream analyses (Danko et al., 2022).Protocol I (Bianco, Calvano, et al., 2022): 9.5 mL of 50 mM Tris-HCl buffer was added to a small aliquot of powdered algae sample (5-8 mg of Spirulina and Chlorella in three replicates).The separation was performed at 20 oC using Dr Maisch precolumn (3.5 cm, 100 um ID precolumn of Reprosil-Pur 120 C18-AQ, 5 um) coupled to a Dr Maisch analytical column (18 cm, 75 um ID analytical column of Reprosil-Pur 120 C18-AQ, 3 um), using water (solvent A) and acetonitrile (solvent B) both containing 0.1 % v/v formic acid.In detail, the gradient used during each chromatographic run, at a flow rate of 250 nL/min, was the following: 0-3 min linear from 1 to 3 % (v/v) of

B, 3–58 min linear from 3 to 35 % (v/v) of B, 58–63 min linear from 35 to 45 % (v/v) of B, 63–66 min from 45 to 100 % (v/v) of B, 66–74 at 100 % of B. The MS parameters were the following: spray voltage, 2.0 kV (positive polarity); capillary temperatures, 275 oC; S–Lens RF Level, 50 (arbitrary units).SDS–PAGE For electrophoresis, each protein extract was resuspended in a sample buffer consisting of 2 % (w/v) SDS, 40 % (v/v) glycerol, 0.02 % (w/v) bromophenol blue, 0.08 M Tris–HCl (pH 8.0), and 10 % Bolt(TM) Sample Reducing Agent.A prestained protein standard was included as a molecular weight marker, and gels were stained with colloidal Coomassie G–250 for protein visualization, following established protocols (Arenas et al., 2021).So, the organic phase was carefully removed and discarded, and the aqueous phase, containing the protein component, was divided into 0.1 mL aliquots, which were dried under a nitrogen flow and preserved.The reducing agent TCEP and the alkylating agent IAA specifically target disulfide bonds, promoting effective protein denaturation while preventing aggregation.Protein quantification The total protein content in the samples was quantified using the Pierce(R) Microplate BCA Protein Assay Kit – Reducing Agent Compatible (Bainor et al., 2011).Pierce Microplate BCA Protein Assay Kit – Reducing Agent Compatible were purchased by Thermo Fisher Scientific.Meanwhile, the detergent SDC plays a pivotal role in disrupting the lipid bilayer, facilitating the solubilization of membrane proteins.Mass spectra processing was conducted using SigmaPlot 14.5.2.2.2.3.2.4.2.5.2.6.2.8.