In-Vivo Lipidomics using Single-Cell Raman Spectroscopy

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ABSTRACT

We describe a method for direct, quantitative, *in vivo* lipid profiling of oil producing microalgae using single-cell laser-trapping Raman spectroscopy (LTRS). This approach is demonstrated in the quantitative determination of the degree of unsaturation and transition temperatures of constituent lipids within microalgae. These properties are important markers for determining engine compatibility and performance metrics of algal biodiesel. We show that these factors can be directly measured from a single living microalgal cell held in place with an optical trap while simultaneously collecting Raman data. Cellular response to different growth conditions is monitored in real time. Our approach circumvents the need for lipid extraction and analysis that is both slow and invasive. Furthermore, this technique yields real-time chemical information in a label-free manner, thus eliminating the limitations of impermeability, toxicity and specificity of the fluorescent probes used in other common protocols. Although the single-cell Raman spectroscopy demonstrated here is focused on the study of the microalgal lipids with biofuel applications, the analytical capability and quantitation algorithms demonstrated are applicable to many different organisms, and should prove useful for a diverse range of applications in lipidomics.

INTRODUCTION

The global concerns surrounding unabated fossil fuel consumption and the risk of significant environmental impact due to the associated greenhouse gas emissions, compounded by potential challenges associated with land-based biofuels[1], have renewed significant interest in microalgae as an alternative feedstock for the production of biodiesel and other biofuels. Microalgae hold considerable promise because of their ability to synthesize and store lipids, such as fatty acids and triacylglycerols (TAGs), that can be readily converted into biodiesel (fatty acid methyl or ethyl esters) through relatively simple chemical reactions[2]. Small yet efficient, microalgae are attractive for many reasons including their (1) rapid, cost-effective, and resource-efficient production including on non-arable land or photobioreactors (PBR)[3], and with impaired water and (2) significant lipid production - up to 20-50% of their total dry weight, with examples of up to 80% under certain conditions reported[4]. It has been estimated that lipid production of microalgae could be 30 times more efficient in terms of relative production of lipids per acre per year than any other terrestrial plant oil feedstock[5, 6].

Under optimal growth conditions, microalgae synthesize fatty acids in the form of various glycerol-based membrane lipids primarily for structural and functional roles[7]. In contrast, under adverse environmental or metabolic stress conditions such as nutrient limitation, also known as "lipid trigger" conditions, lipid biosynthetic pathways in many microalgae are hypothesized to be upregulated in favor of the formation and accumulation of substantial proportions of neutral lipids (20 - 50% of dry weight), mainly in the form of TAGs. These TAGs are a form of efficient energy storage within the microalgae, stored as dense lipid bodies. It is also reported that the lipid trigger conditions result in low growth rates for the algae, thereby placing an upper limit on

the total lipid production that can be achieved under these conditions[5, 8]. Novel strain selection, genetic and physiological manipulations may offer solutions that shift this balance between growth rate and lipid biosynthesis, but require facile methods that establish correlations between lipid trigger conditions, growth rate, and lipid production.

Lipid content within microalgae, as in the general field of lipidomics[9] , is commonly analyzed using solvent extraction based methodologies, with the lipids subsequently quantified gravimetrically[10-12]. Further separation and analysis of lipids can be carried out using chromatography (GC or HPLC)[13], mass spectrometry[14], or NMR[15]. These steps provide compositional identification of the extracted lipids but are relatively time and effort-intensive. Moreover, the information linking cellular dynamics and functionality is often lost during the homogenization step typically required for these assays, and therefore they become less useful for understanding and controlling the fundamental biological processes needed for developing physiological manipulation and metabolic engineering methods. Staining microalgae cells with an environmentally-sensitive lipid-soluble fluorescent probes (e.g., Nile Red) that can discriminate between neutral and polar lipid environments has been shown to allow single-cell lipid analyses, including high-throughput techniques[16, 17]. While useful, these approaches are limited by the availability of photostable fluorescent lipid probes with a well-characterized partitioning preference. For some species of microalgae these fluorescent probes can be toxic or impermeable, and their observation can be confounded by the auto-fluorescence from photosynthetic pigments such as carotenoids[18, 19](**Supplementary Fig. 1**). Moreover, these approaches provide little chemical information about the lipids, such as the composition, degree of unsatuaration, and chain length. Since these chemical characteristics ultimately determine the

quality of the derived biofuel, including viscosity, cloud point, burning efficiency, cetane rating and stability against oxidation and polymerization[20], alternative approaches that offer direct and rapid quantification of lipid properties are highly desirable.

Raman spectroscopy offers an attractive alternative for deriving direct quantitative chemical information in a label-free, nondestructive, and real-time manner at the single cell level without requiring any exogenous modification of samples[21]. Specifically, laser-trapping Raman spectroscopy (LTRS), a combination of a near-IR optical trap[22] and a micro-Raman system[23, 24], is particularly well-suited to meet these requirements. It produces *in vivo* spectra from single living cells with higher signal-to-noise ratios than conventional Raman measurements performed on bulky and dried algal samples[25, 26] and enables a new level of quantitative analysis. As demonstrated in this work, after building a Raman spectral library of model microalgal lipids that can be used to readily determine quantitative information such as the degree of unsaturation (quantified by the average number of C=C bonds per lipid molecule), lipid chain length, and melting temperature (T_m) of the constituent fatty acids in microalgae can be performed on a single living cell. We have obtained and interpreted *in vivo* single-cell Raman spectra of several algal species of interest to bioenergy, including *Botryococcus braunii* (*B. braunii*), *Neochloris oleoabundans* (*N. oleoabundans*) and *Chlamydomonas reinhardtii* (*C. reinhardtii*). Our approach of interrogating single cells for information related to growth and lipid production should not only pave the way for direct and rapid screening of microalgae, but also enable the optimization and selection of species and growth conditions and rapidly test the efficacy of physiological and genetic manipulations.

RESULTS

Experimental setup

The single-cell LTRS setup combines an optical trap and a micro-Raman system with a confocal pinhole setup (Online Methods) in a single custom-built configuration. The micro-Raman system consists of a 785-nm laser (also used for the optical trap), an inverted microscope with a 60X 1.2 numerical aperture (NA) lens fitted with a filter set to clean the laser and block Rayleigh scattering, a spectrograph, and a CCD detector (**Fig. 1**). For samples of microalgae suspended in their native growth media or other aqueous buffer, the laser focused by the high NA objective forms an optical trap that immobilizes a cell and also allows lifting it several micrometers from the bottom to avoid surface-induced perturbations. Signals from regions outside the sample point are rejected by the confocal pinhole affording high signal-to-noise ratio needed for single cell spectroscopy. Since a trapped cell is in a large reservoir of aqueous buffer, the temperature within the trap is stable near the bath temperature[27]. As no loss of activity of a biflagellate *C. reinhardtii* cell has been observed after a 10-minute exposure in the laser trap, this system is thought to have a minimal negative impact on the state of health of the cells (**Supplementary Video 1**). Typical acquisition times for well-resolved spectra of pure fatty acids were found to be less than one second and for a microalgal cell less than 10 seconds (Online Methods).

Raman spectral library of algal model lipids

A spectral library was assembled from Raman spectra of several single fatty acids typically found in algal lipid extracts (**Fig. 2**). Several interrelated diagnostic Raman markers, directly associated with lipid unsaturation and their phase state deduced from the data in Fig. 2 are used to guide the analyses. ***First***, Raman bands corresponding to (1) =C-H *cis* stretch at 1260 cm^{-1}; (2) C=C stretch at 1650 cm^{-1}; and (3) =C-H stretch at 3023 cm^{-1} provide unequivocal evidence

for chain unsaturation. ***Second***, the presence and relative intensities of C-C *gauche* stretch at 1075 cm^{-1} and C-C *trans* stretches at 1056 cm^{-1} and 1116 cm^{-1} can be used to assess the phase state of the constituent lipids. This is because fatty acid tails are packed in an orderly *trans* conformation in the solid phase[28] whereas *gauche* conformers populate the more disordered, fluid phases. ***Third,*** peaks assigned to saturated CH_2 bonds, such as CH_2 twist at 1300 cm^{-1}, CH_2 bend at 1440 cm^{-1}, and CH_2 symmetric and asymmetric stretches at around 2800-3000 cm^{-1}, are typically strong in saturated fatty acids. For instance, between arachidonic acid (20:4, T_m = -50 °C) and stearic acid (18:0, T_m = 70 °C), the unsaturation markers, 1260 cm^{-1}, 1650 cm^{-1} and 3023 cm^{-1} consistently diminish, while the relative intensities of the bands related to the CH_2 bond, increase. The *gauche* conformers are gradually suppressed and intensities due to *trans* conformers increase for lipids with higher melting temperature. These markers provide a semi-quantitative classification for the presence and concentration of specific lipid types in unknown samples. Semi-quantitative information related to the chain length, unsatuation, and melting temperature can be deduced from the Raman spectra. This information is inaccessible in fluorescence-based data.

In vivo Raman spectra of microalgal cells

Single-cell LTRS delivers a high-quality Raman spectrum of a single cell that is held steady in a laser trap. Representative LTRS spectra of single cells of several strains, *N. oleoabundans* cell culture in nitrate-depleted media, and lipids extracted from *N. oleoabundans* are shown in **Fig. 3**. The extracted microalgal lipids show all major lipid Raman bands, which are also represented in the microalgae cells. Raman spectra can also identify other important components such as proteins, carbohydrates, and pigments (**Table 1**). Information about proteins can be obtained

from the amide I and III bands (1600-1700 cm^{-1} and 1200-1350 cm^{-1}, respectively) and from the distinctive phenylalanine symmetric stretching (ring breathing) at 1004 cm^{-1}. In addition, carotenoid characterized by bands at 1008 cm^{-1}, 1160 cm^{-1} and 1537 cm^{-1}, and cellulose, seen at 479 cm^{-1}, 945 cm^{-1}, 1082 cm^{-1} can help provide insights into queries related to algal biology.

A comparison of these *in vivo* single-cell Raman spectra of *B. braunii* and *N. oleoabundans* with the spectra of other types of biomass, such as terrestrial plants[29], bacteria[30] and human cells[24], shows the lipid signatures (e.g., 1440 and 1650 cm^{-1}) of the microalgae to be substantially stronger than other components, confirming high lipid content in the microalgae cells. To determine the ability of Raman spectroscopy to characterize alterations of the lipid profiles within single cells following a change of the their environment, the *N. oleoabundans* cells were stimulated by culturing in nitrate-depleted media and observed by single-cell LTRS. The overall spectral signatures reveal a significant increase in bands attributed to the lipids under nitrate-depleted conditions, confirming a net increase in the lipid content. Although this increase in lipid content, especially TAGs, can be monitored by fluorescence methods using a cellular suspension of the same culture (**Supplementary Fig. 2**), the single-cell LTRS provides additional information regarding the chemical characteristics of these compositional changes. The spectra indicate an obvious increase in the overall lipid content (e.g., enhancement of 1440 cm^{-1}, 1736 cm^{-1} and 2820-3030 cm^{-1} bands), that occurs concurrently with a significant decrease in carotenoid and protein content. This is evident from the relative intensities of the carotenoid associated bands (e.g., 1008, 1160 and 1537 cm^{-1}) and the protein-associated amide bands (e.g., 1004 cm^{-1}, amide I and amide III band) (see **Table 1** for band assignments). This finding is

consistent with the notion that, within single cells, nitrate starvation induces an increase in the total lipids present per cell[31].

Quantitative analysis of lipid unsaturation

In addition to the qualitative information determined above from the single-cell Raman spectra, quantitation is also possible. Beer's law indicates that the spontaneous Raman scattering is linearly dependent on the analyte concentration[32, 33]. This relationship indicates that by measuring relevant experimentally acquired Raman bands assigned to specific lipid types (or proteins), concentrations of the corresponding molecular species can be estimated. In actual practice, however, the inherent irreproducibility in condensed phase Raman spectroscopy measurements limits the direct applicability of Beer's law for absolute determination of concentrations from single peaks. The use of ratiometric analyses involving a comparison of Raman intensities of two independent, but molecularly related, peaks in the same spectrum circumvent this limitation. They also provide important diagnostic markers. For instance, the ratio of Raman intensity of the C=C stretch and the intensity of CH_2 bend, I_{1650}/I_{1440}, displays a linear dependence (R^2=0.99) with the ratio of the number of C=C bonds and the number of CH_2 bonds for the model lipids (**Fig. 4a**). The relationship is also linear (R^2=0.99) when the ratio I_{1650}/I_{1440} is plotted simply as a function of the number of C=C bonds per lipid molecule (**Fig. 4b**). This approach also eliminates spectral artifacts due to source intensity fluctuations, sample-to-sample variabilities, and other uncontrolled experimental parameters, thus yielding reliable quantitative information.

This ratiometric method provides a quantitative means with which to estimate the average number of C=C bonds when the ratio of I_{1650}/I_{1440} can be measured. For example, I_{1650}/I_{1440} obtained from the lipids extracted from *N. oleoabundans* in normal growth condition and from the triggered individual cells are plotted on the graphs shown in **Fig. 4a** and **4b** (see **Supplementary Note 1** for fitting parameters). These results suggest that the extracted algal lipids have, on average, approximately 1.25 double bonds per lipid molecule and that the double bond to CH_2 bond ratio is approximately 0.124. For the lipids in triggered cells, there are approximately 0.97 double bonds per lipid molecule on average, and the double bond to CH_2 bond ratio is approximately 0.09. Sharing similar formula $CH_3(CH_2)_m(CH=CH)_nCOO$-, the average chain length of the fatty acids incorporated in the TAGs equals the number of CH_2 bonds plus two carbons in each C=C bond and two carbons at the COO- head and -CH_3 tail, e.g., , $1.25/0.124 + 2\times1.25 + 2 \approx 14.6$, for the extracted algal lipids. Therefore, based on the ratiometric data summarized above, the average fatty acid chain formula of the lipids extracted from the normal *N. oleoabundans* cell estimates to be 14.6:1.25, which matches the length of the fatty acid methyl easter reported using gas chromatography[13].

LTRS measurements show that for the individual cells cultured in nitrate-depleted media, the average chain length is shortened to n = 14.3, with a decreased degree of unsaturation of approximately 0.97 C=C bond per lipid. This difference in the degree of unsaturation associated with the lipid makeup of whole cells in nitrate-depleted media is presumably caused by the lipid synthetic pathways in response to the stress induced by nitrate-depleted growth condition. This finding is consistent with many reports of the lipid profile changes that occur in response to growth conditions, such as nutrient limitation[14, 34-38].

In situ measurements of lipid melting temperature

Further insight can be gained from the fact that the degree of unsaturation is directly related with the melting temperature (T_m) of fatty acids, another critical factor affecting the quality of biodiesel. Temperature dependence of chain order parameters typically generate sigmoidal curves[39, 40], which can be used to predict the T_m of unknown fatty acids using Raman spectroscopy. Specifically, T_m of the model fatty acid relates to the unsaturation that is quantified by Raman ratio I_{1650}/I_{1440}, and can be fitted with a sigmoidal curve (R^2=0.98) (**Fig. 4c**) (see **Supplementary Note 1** for fitting parameters). The Raman ratio I_{1650}/I_{1440} from the lipids extracted from *N. oleoabundans,* and from the lipids in individual nitrate-starved cells are then plotted on the same calibration curve, predicting a transition temperature of approximately -0.3 °C for the extracted lipids and 2.6 °C for the lipids measured in whole cells. When the T_m of the extracted lipids was measured by differential scanning calorimetry (DSC), a difference of less than 1 °C separated the predicted and measured values (**Fig. 4d**). As DSC usually requires milligrams of sample for each measurement, obtaining this level of accuracy from single cells represents a significant advance in characterizing the lipids within microalgae. These results demonstrate unprecedented real-time *in situ* quantitative oil profiling and melting temperature prediction within a live cell during growth and oil production.

DISCUSSION

The enormous biodiversity (200,000-800,000 species) of microalgae poses huge challenges in the identification and screening of potential candidates for effective biofuel production[41, 42]. The utilization of genetic manipulation and physiological engineering to improve biofuel production

only increases the sample diversity. This study of only five algal species in response to changes of light exposure, nitrogen, silicon, CO_2, pH, and temperature required culturing a minimum of 30 samples. This number rapidly increases when full factorial statistically designed experiments are required to understand the important biotic and abiotic factors affecting growth and lipid production. Central to overcoming these challenges is the development of a rapid, facile, and reliable analytical method to characterize the composition and concentration of energy-rich neutral lipids. The work reported here illustrates the promise of single-cell Raman spectroscopy for a rapid, accurate, and quantitative characterization of lipid profiles from single microalgae cells in a non-destructive and label-free manner. The technique is amenable to high-throughput screening and/or a sorting-based assay when coupled with microfluidics, or it can be implemented as a pond- or bioreactor-based *in situ* screening assay for the routine assessment of algal health and productivity. Microalgae have also been utilized as a resource for obtaining other compounds, such as dietary fatty acids and proteins, as well as ingredients of cosmetics and pharmaceuticals[43], such as antioxidant carotenoids, which also have distinct Raman signatures. Single-cell Raman spectroscopy would also be useful for *in situ* and direct monitoring of these components. Thorough quantitative analysis enabled by chemometrics techniques[44] could extract more information from lipids and other important components produced in microalgae.

The use of single-cell LTRS affords a simple, quick and highly effective method to interrogate individual microalgal cells with the high specificity of information, and promises to enable rapid progress on the road to sustainable, as well as economically and environmentally responsible, energy supplies. This demonstration of facile label-free quantitative *in-vivo* analysis of microalgae is suitable to understanding lipid profiles of any organism or metabolic system where knowledge of fast dynamics of metabolites, pathways and lipid compositions are desired. Lipid

metabolism disorders and their link to numerous diseases have led to a dramatic increase in research and system-level studies of extremely intricate cellular interactions and lipid profiles. Mass spectrometry and chromatography based techniques on cell extracts have filled significant gaps in our current understanding in terms of lipid metabolism disorders. To date, there are a handful of experimental approaches using living cells utilizing fluorescent lipids that have also shown promise, but are limited in its ability due to non-specificity providing mostly qualitative signatures [45]. This *in vivo* capability expands the list of available analytical tools to researchers in the emerging field of lipidomics for multiple applications[9, 46].

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AUTHOR CONTRIBUTIONS

H.W. designed and performed experiments, and analyzed the data. J.V.P. maintained, monitored algal cell cultures and conducted Nile Red analysis. A.E.O and A.N.P. conducted the DSC measurements. S.S. and B.A.S. conceived and supervised the project. H.W. and S.S. wrote the manuscript. A.N.P., A.E.O. and B.A.S. discussed the results and edited the manuscript.

FIGURES AND CAPTIONS

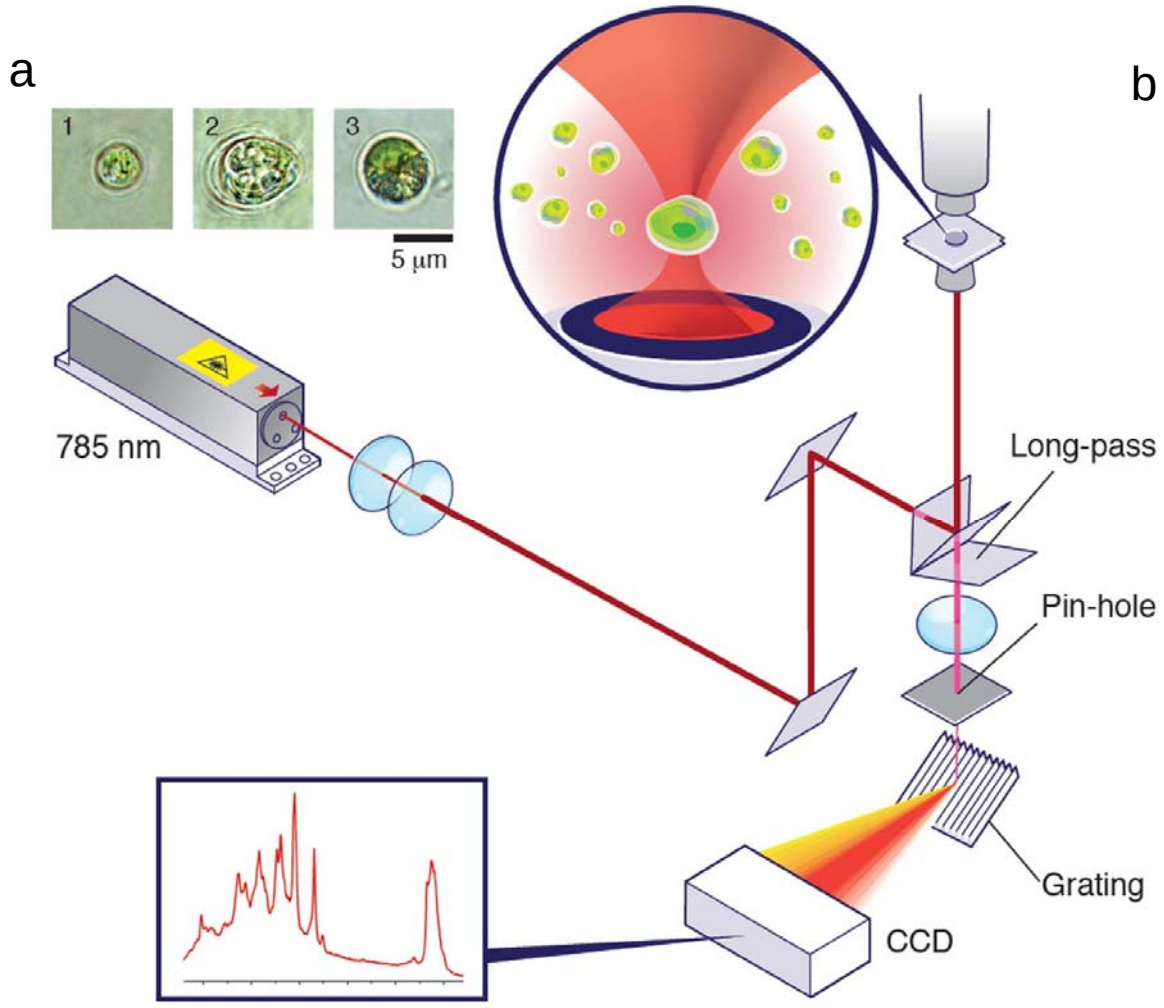

Figure 1 Single-cell laser-trapping Raman spectrosocpy (LTRS) setup. (a) Individual microalgal cells immobilized by the optical trap, imaged by bright-field microscopy: (1) *N. oleoabundans;* (2) *B. braunii;* and (3) *C. reinhardtii.* (**a**) Instrument layout showing the 785 nm laser beam used as both trap beam and Raman excitation beam, excitation path, Raman scattering path, positions of objective lens, pin-hole, grating and CCD.

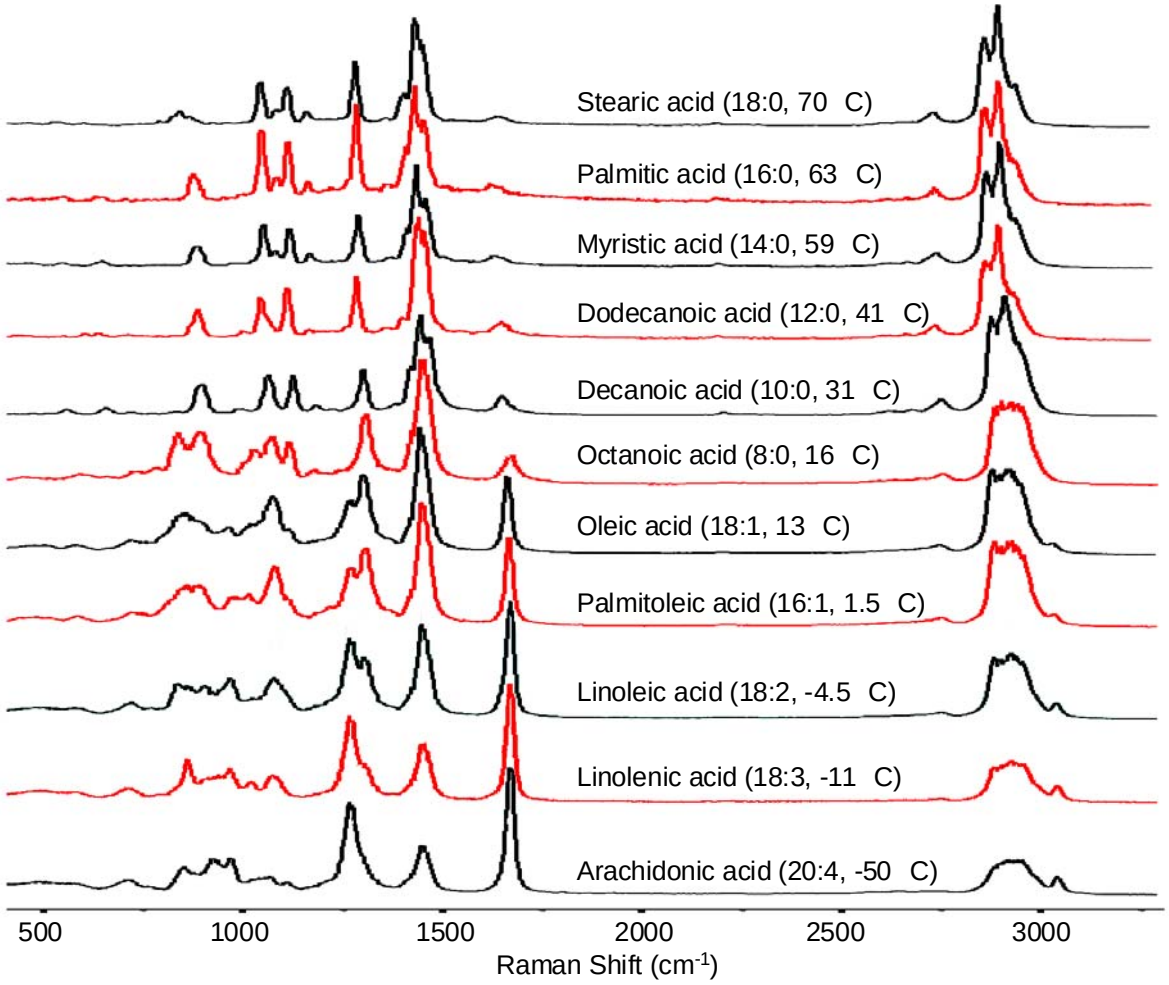

Figure 2 Raman spectra library of 11 fatty acids, listed in descending order of their respective melting temperatures (T_m).

Figure 3

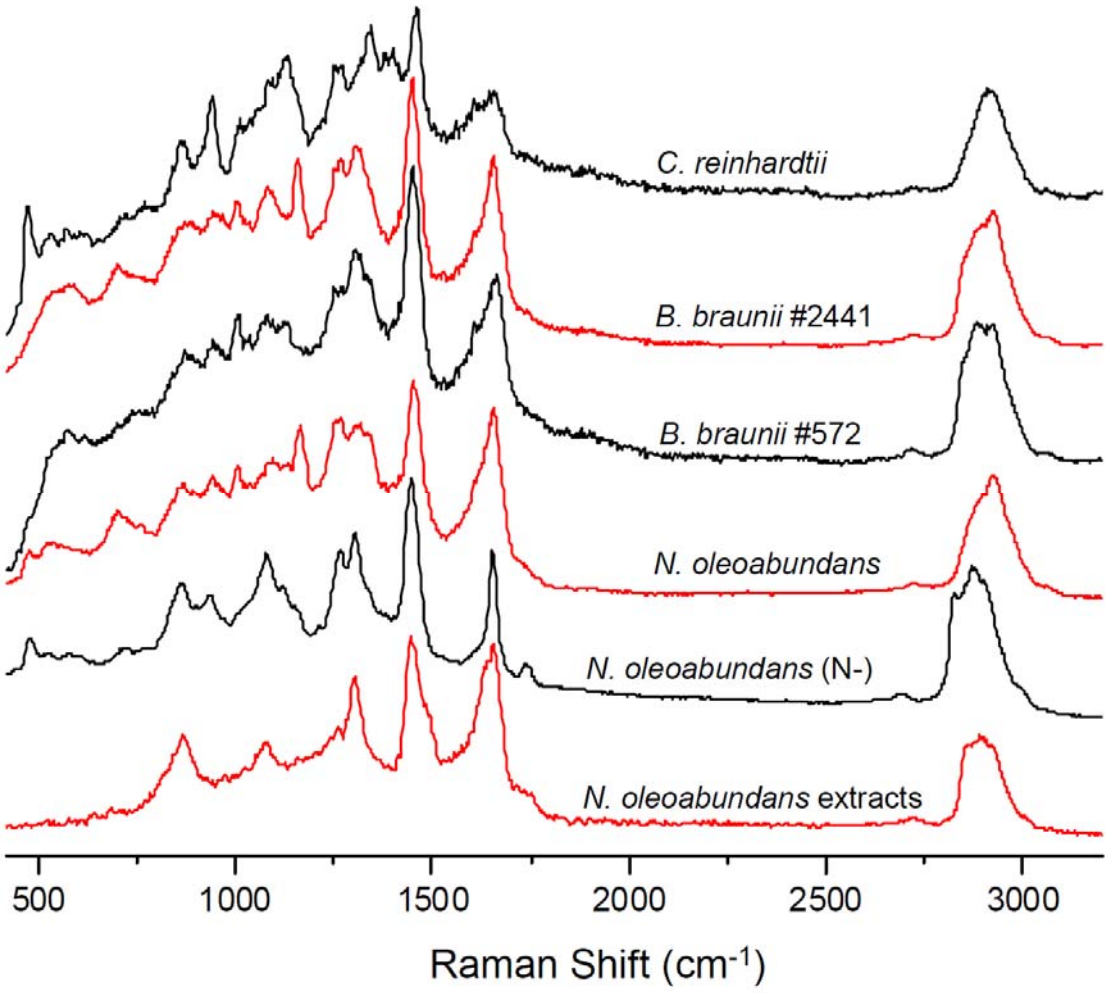

Figure 3 *In vivo* single-cell LTRS spectra of several species of microalgae, from top to bottom: *C. reinhardtii*, *B. braunii* (UTEX# 2441), *B. braunii* (UTEX# 572), *N. oleoabundans* and *N. oleoabundans* grown in nitrate-depleted media (N-), and the spectrum of algal lipids extracted from normal *N. oleoabundans* by chloroform/methanol method..

Figure 4

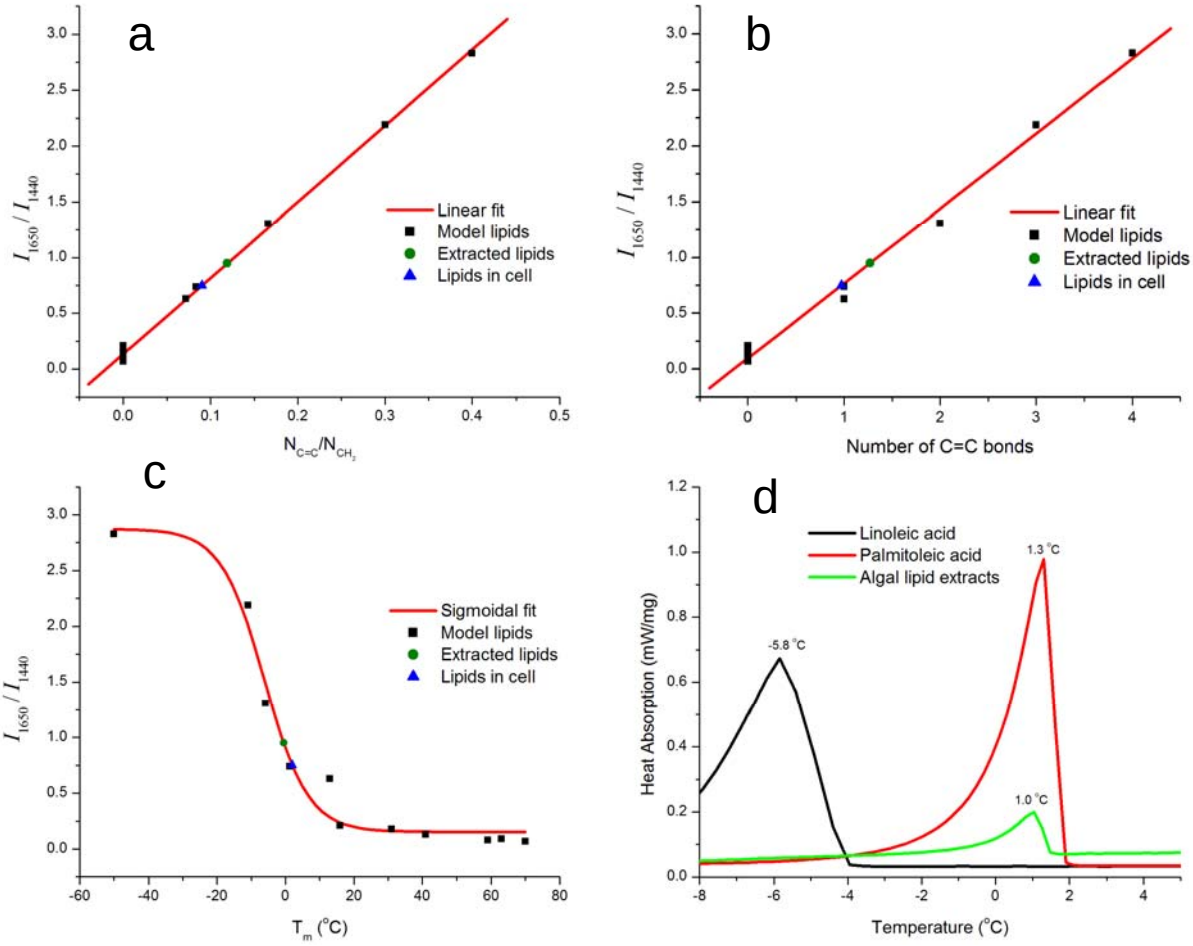

Figure 4 Quantitative analysis of degree of unsaturation, chain length and melting temperature of fatty acids by Raman spectroscopy, and validation by DSC method. (**a**) The ratio of the intensity of the 1650 cm^{-1} band (C=C stretch) to the 1440 cm^{-1} band (CH_2 twist), I_{1650}/I_{1440} of fatty acids standard are plotted versus the ratio of the number of C=C bonds to CH_2 bonds, and fitted linearly (R^2 =0.99), then I_{1650}/I_{1440} measured on the lipids extracted from normal *N. oleoabundans* cells and the lipids in the individual *N. oleoabundans* cells grown in nitrate-depleted media is fitted on the curve to predict their molecular structure. (**b**) I_{1650}/I_{1440} are plotted verses the number of C=C bonds per molecule and fitted linearly (R^2 = 0.99), to predict degree of

unsaturation of the lipids extract from normal *N. oleoabundans* cells and the lipids in the individual *N. oleoabundans* cells grown in nitrate-depleted media. **(c)** I_{1650}/I_{1440} of fatty acid standards are plotted versus their published melting temperatures and fitted with a sigmoidal Boltzmann function ($R^2 = 0.98$), to predict the melting temperatures of the lipids extracted from normal *N. oleoabundans* cells and the lipids in the individual *N. oleoabundans* cells grown in nitrate-depleted media. (**d**) DSC measurement of the melting temperature of the lipids extracted from normal *N. oleoabundans* cells confirms the prediction based on LTRS Raman spectra.

TABLES

Wavenumber (cm^{-1})	Components	Assignments
479	Carbohydrate	C-C-C deform.
744	Carbohydrate, chl*a*,	H–C–O str. and chl*a* N-C-C deform.
854	Carbohydrate	Hemiacetal str. and methylene deform.
865	Phospholipid	C_4N^+ and O-C-C-N sym. str.
945	Carbohydrate, protein	α-helix C−C bk. str., C−O−C str.
1004-1008	Protein, carotenoid	Phenylalanine ring breath, carotene C-H bending
1056, 1116	Lipid	Alkyl C-C *trans* and *gauche* str.
1075	Lipid	Alkyl C-C *gauche* str.
1082	Carbohydrate	Carbohydrate C-O-H bending
1120	Carbohydrate	C-O-H deform., C-O and C-C str.
1160	Carotenoid	Carotene C-H str.
1200-1350	Protein	Amide III
1260	Lipid	Alkyl =C-H *cis* str.
1300	Lipid	Alkyl C-H_2 twist
1340	Carbohydrate, chl*a*	Carbohydrate C-H_2 deform. and C-O-H bending, Chl*a* C-N str.
1440	Lipid	Alkyl C-H_2 bend
1537	Carotenoid	Carotene C=C str.
1600-1700	Protein	Amide I
1650	Lipid	Alkyl C=C str.
1736	Lipid	Ester C=O str.
2850-2930	Lipid, carbohydrate	C-H_2, C-H_3 asym. and sym. str.
3023	Lipid	Alkyl =C-H str.

Table 1 Assignments for Raman bands (medium to strong bands only), collected from the following references: carotenoid[47], chlorophyll *a* (chl*a*)[48], lipids[23], protein[49], and carbohydrate (including cellulose)[29, 50]. Deform. = deformation, str. = stretches, bk. = backbone, sym. = symmetric, asym. = asymmetric.

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ONLINE METHODS

Algal strain, culture condition and lipid triggering

Botryococcus braunii (UTEX #572 and #2441) with its growth medium, Bold 3N, and *Neochloris oleoabundans* (UTEX #1185) with its growth medium, Soil Extract, were obtained from the Culture Collection of Algae, at the University of Texas at Austin. *Chlamydomonas reinhardtii* (CC-503 cw92 mt+) and Sueoka's growth medium were obtained from the Chlamydomonas Center at Duke University. Algal cultures of 120 mL volume were grown in sterile 250 mL glass culture flasks covered loosely with sterile foil. Culture flasks were placed in a diurnal incubator at a temperature of 20.8 ^{o}C with a daily 24-hour cycle of 16 hours fluorescent light followed by 8 hours of dark.

Nitrate-depleted growth of *N. oleoabundans* cultures were obtained by initially growing cultures in Soil Extract medium. At day 19 of growth, the regular medium was changed to the triggering medium, which was made by following the soil extract recipe from UTEX, but eliminating the $NaNO_3$ component entirely. The other components included $CaCl_2{\cdot}2H2O$, $MgSO_4$ (anhydrous), K_2HPO_4, KH_2PO_4, NaCl and Soil-Water Medium purchased from WARD'S Natural Science (Rochester, NY).

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise stated. Organic solvents are all HPLC grade.

Lipid extraction

Chloroform methanol extraction of *N. oleoabundans* is based on the method described by Bligh and Dyer (Bligh_1959) with slight modifications. A mixture of chloroform and methanol (2:1 v/v) was used as an extraction solvent. Glassware was used for all the steps to minimize polymer contamination. One liter of *N. oleoabundans* culture at confluence was used. Confluence was reached after 16 days of growth following triggering. The cells were centrifuged at 4000 RPM for 10 minutes. The cell pellets were then collected and resuspended in 1 mL DI water in a glass vial. The vial was sonicated for 10 min in a 50 °C water bath. 3 mL of extraction solvent was added into the vial. The extraction step was processed by placing the vial in a 50 °C water bath for 1 hour while vortexing vigorously for 1 minute every 10 minutes, and allowing the suspension to settle for 30 sec on ice in between each vortex. Afterwards the sample was placed at -20 ^{o}C for 1 hour to accelerate separation. After separation, the bottom oil/chloroform phase was transferred with a needle, and stored at -20 ^{o}C for future use. To evaluate lipids by Raman measurements, the chloroform was evaporated under a stream of nitrogen and the sample was placed under vacuum for 2 h to remove residual chloroform. The lipid film was finally resuspended in DI water at a concentration of 5 mg/mL.

Single-cell Laser-trapping Raman Spectroscopy

Briefly, a 785 nm laser with 70 mW output power from a continuous wave DPSS laser (CrystaLaser, Reno, NV) was directed through a 4X beam expander, and a 785 nm bandpass filter (Omega Filters, Brattleboro, VT) to remove any possible plasma emission generated within the laser tube. The beam was then delivered into an inverted Olympus optical microscope (IX71), which is equipped with a dichroic longpass beamsplitter (Chroma Technology, Rockingham, VT) to reflect the laser beam into a 1.2 N.A. 60X water immersion objective lens,

resulting in a diffraction limited spot of 0.5 μm diameter at the laser focus. The beam was focused through a quartz coverslip, with a power of approximately 10 mW out of the objective. Raman scattering generated at the focus were collected by the same objective, passed through the dichroic beamsplitter and a long-pass edge filter (Omega Filters, Brattleboro, VT) to block elastic scattering photons, and then focused through a 100 μm pinhole for background signal rejection. The signal is dispersed by a spectrometer (Acton #2300i, Princeton Instruments, Trenton, NJ) equipped with 1 μm blaze wavelength 300 g/cm grating and projected on an air-cooled EM-CCD with 1340 × 100 pixels (PIXIS, Roper Scientific, Tucson, AZ). Spectral resolution was higher than 5 cm^{-1}.

For measurements of fatty acid standards, a small amount of sample (5 μL for liquid samples or a couple of grains for solid samples) were put on the cleaned quartz coverslip. The focal point of the laser was moved onto the sample. Measurements were done at room temperature. The acquisition time was 1 second for fatty acid standards.

For particles suspended in aqueous buffer, the laser focused by the high N.A. objective lens forms an optical trap that can immobilize a single micron-sized particle. The focused laser can trap the particle and lift it several microns above the surface, which reduces surface perturbation. In addition, noise from regions other than the focal point is rejected by the confocal pinhole. For both these reasons, the LTRS has an extremely high signal-to-noise ratio.

For measurements of single cells, cell suspension was diluted, such that only a few cells would occupy the microscopic field of view. Sample (40 μL) was placed on the cleaned quartz

coverslip. Measurements were started 2 min after a cell was trapped, enabling the prerequisite photobleaching of the chlorophyll fluorescence, and acquisitions were made for 10 s. Raman spectra were acquired and processed by WinSpec32 software (Roper Scientific, Tucson, AZ). After background subtraction, the spectra are smoothed by employing an averaging filter. The intensity of each peak was calculated by subtraction of the average baseline value of that peak from the maximum intensity value. Baselines for each peak were simplified as a line connecting the two lowest points at the left and right sides of the peak. Data fitting was done by Origin software (Microcal Software, Inc., MA)

DSC Measurements

Free fatty acid standards, linoleic acid and palmitoleic acid, were tested as received without further purification. A small volume (30 μL) of each was sealed into separate DSC crucibles. For the algal extracts, approximately 1.5 mg of oil was evaporated from chloroform under a stream of N_2 directly into the DSC crucible. Residual chloroform was removed under vacuum for at least 24 h before sealing the crucible. Phase transitions were measured by differential scanning calorimeter using a Mettler Toledo DSC822e. Samples were scanned from -20 or -30 ^{o}C to 20 ^{o}C, initially at 10 ^{o}C/min, and on subsequent scans at 1 ^{o}C/min. The values reported were taken at 1 °C/min. Lipid phase transitions were taken as the maxima of endothermic peaks on thermal upscans.