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Understanding the lipid production mechanism in Euglena gracilis with a fast-response AIEgen bioprobe, DPAS

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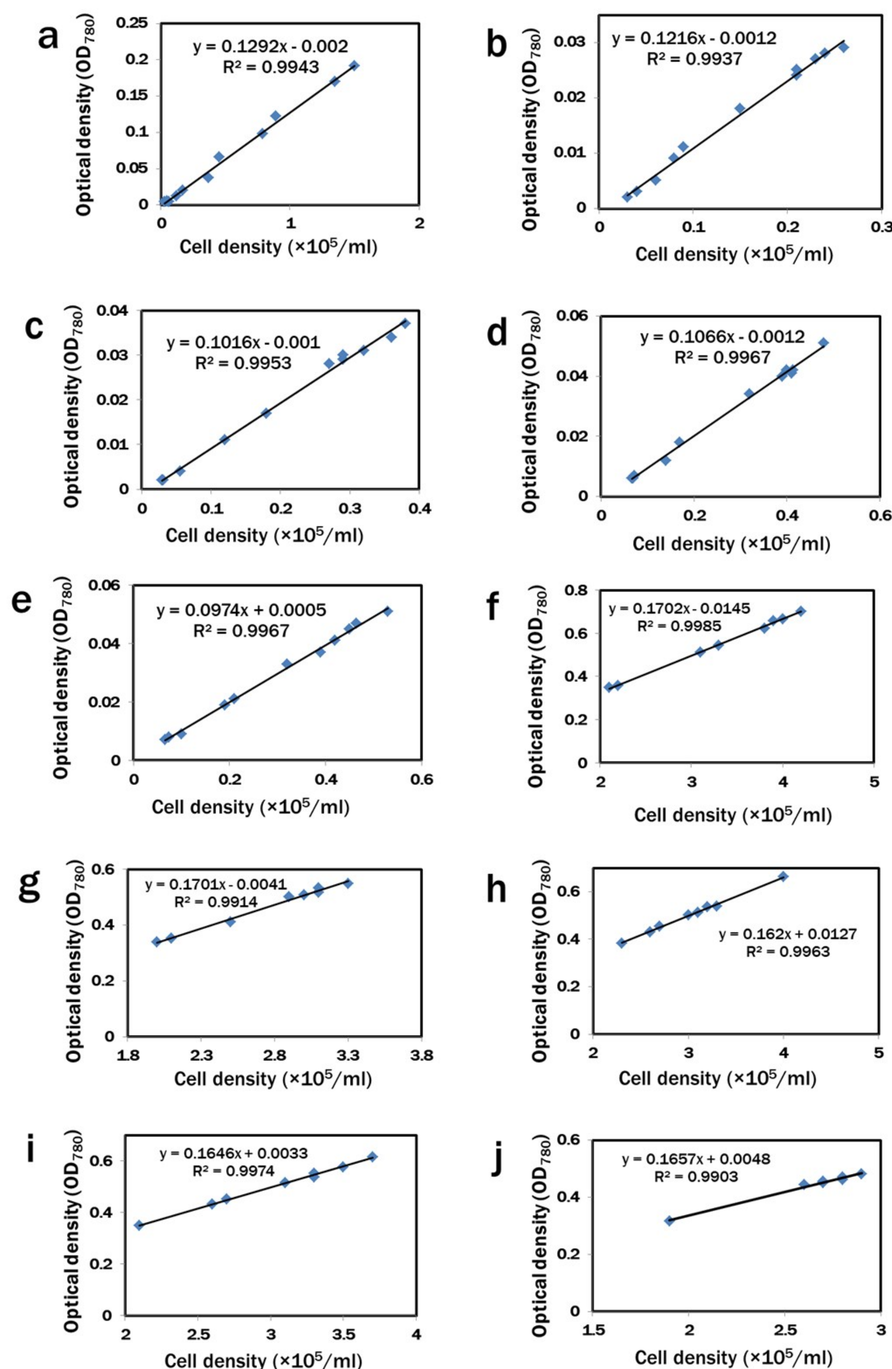
Supplementary Information

Supplementary S1- Linear relationship between OD_{780} and cell density cultured in (a, f) Treatment 1: Modified Cramer-Myers medium (MCM); (b, g) Treatment 2: MCM, (-) N_2; (c, h) Treatment 3: MCM, (-) N_2, (-) Ca^{2+}; (d, i) Treatment 4: MCM, (-) N_2, (-) Ca^{2+}, (+) Glucose (24 h light); (e, j) Treatment 5: MCM, (-) N_2, (-) Ca^{2+}, (+) Glucose (24 h Dark) conditions. (a-e) culture initiated with 0.05 ± 0.02 (× 10^5) cells/ml; (f-j) culture initiated for lipid induction with 2.08 ± 0.14 (× 10^5) cells/ml.



Supplementary S2- Autofluorescence properties of *E. gracilis*

Due to the presence of different intracellular pigments, microalgae often show strong autofluorescence that hinders proper utilisation of fluorophores for studying target molecules. To select the appropriate dyes for the study in spectrophotometry and confocal microscopy, and to minimise the background noise, the spectral profile of the autofluorescence from live *E. gracilis* cells was determined in this study.

S2-1: Spectrophotometric analysis

Algal cells excited at 350 nm emitted weak fluorescence at around 460 nm (Fig. a), whereas cells excited at 405 nm showed two emission peaks at around 460 nm and 700 nm (Fig. b). Excitation of the cells at 488 nm and 560 nm resulted in a single emission peak at 700 nm (Fig. c and d). The emission around 460 nm was assumed to be due to the number of redox ratios ((NAD(P)H/FAD)), which was directly related to the cellular metabolic activity.[S1] The emission at 700 nm was due to the autofluorescence from chlorophyll, since no fluorescence was detected from the heat-treated dead cells with excitation at 488 nm (Fig. e). As shown in Fig. e, Treatment 1 with CM medium under light condition resulted in the maximum amount of chlorophyll, whereas the condition of N deficiency and dark resulted in the lowest chlorophyll.

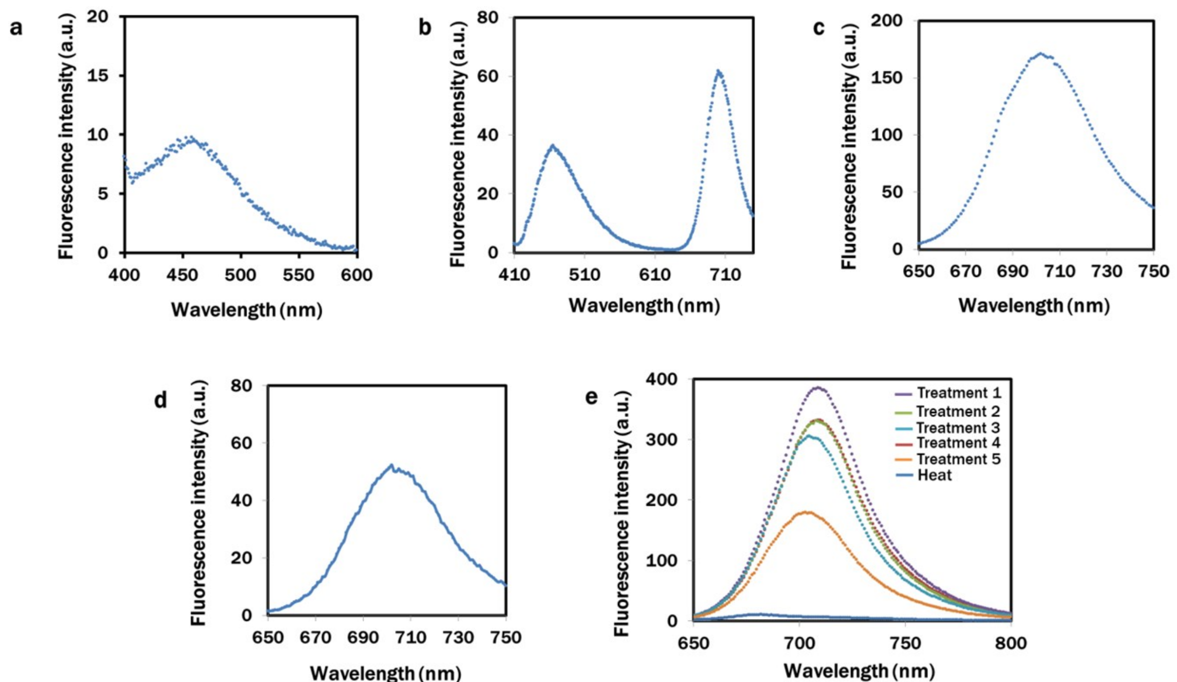


Fig. Autofluorescence of *Euglena gracilis* excited at different excitation wavelengths (a) excition at 350 nm; (b) excitation at 405 nm; (c) excitation at 488 nm; (d) excitation at 560 nm; (d) autofluorescence of chlorophyll from different treatments excited at 488 nm

S2-2: Confocal microscopy

Images from the confocal microscopy also showed that excitation of the cells with laser light at 405 nm resulted in emission at 450-500 nm (Fig. b; Supplementary Movie M1, ESI), whereas no fluorescence was detected at 450-500 nm, when the cells were excited at 488 nm (Fig. f). At both excitation wavelengths, autofluorescence from chlorophyll was detected at 680-760 nm (Fig. c and g). In spectrophotometry results, the maximum autofluorescence for chlorophyll occurred while the cells were excited at 488 nm. Therefore, the chlorophyll fluorescence from different treatments was measured at 488 nm excitation.

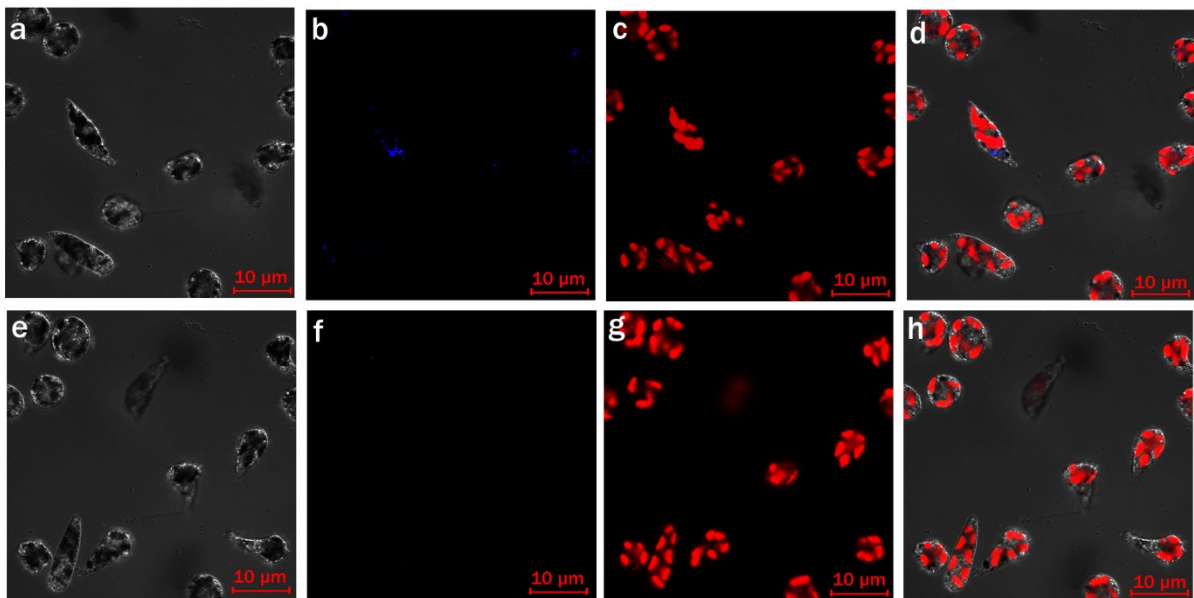


Fig. Autofluorescence of *Euglena gracilis* under Zeiss LSM 880 Airyscan confocal microscopy at λ_{ex} = 405 nm (a-d), and λ_{ex} = 488 nm (e-h). Brightfield (a, e); λ_{em} = 450-500 nm (b, f); Chlorophyll (λ_{em} = 680-760 nm, c, g); Merge (d, h)

Supplementary S3- Fluorescent properties of DPAS

The absorption and photoluminescence (PL) spectra of DPAS (10 μM) in DMSO under the excitation of 405 nm were determined. DPAS showed weak emissions in DMSO solution, but the PL intensity began to increase with the addition of water in DMSO. The emission increased rapidly, while the water percentage increased from 80% to 90%, signifying the AIE attributes of DPAS (Fig. a and b). Although the fluorescence intensity of DPAS in the presence of *E. gracilis* cells at 10 min was slightly lower than that of the incubation after 30 min, 60 min, and 120 min, the result was not significantly different. Therefore it was assumed that DPAS could completely label lipid drops in cells within 30 min (Fig. c). The aggregation of DPAS in the lipid was also studied with the confocal microscope using commercially available 2% sunflower oil (Fig d-f; Supplementary Movie M2, ESI).

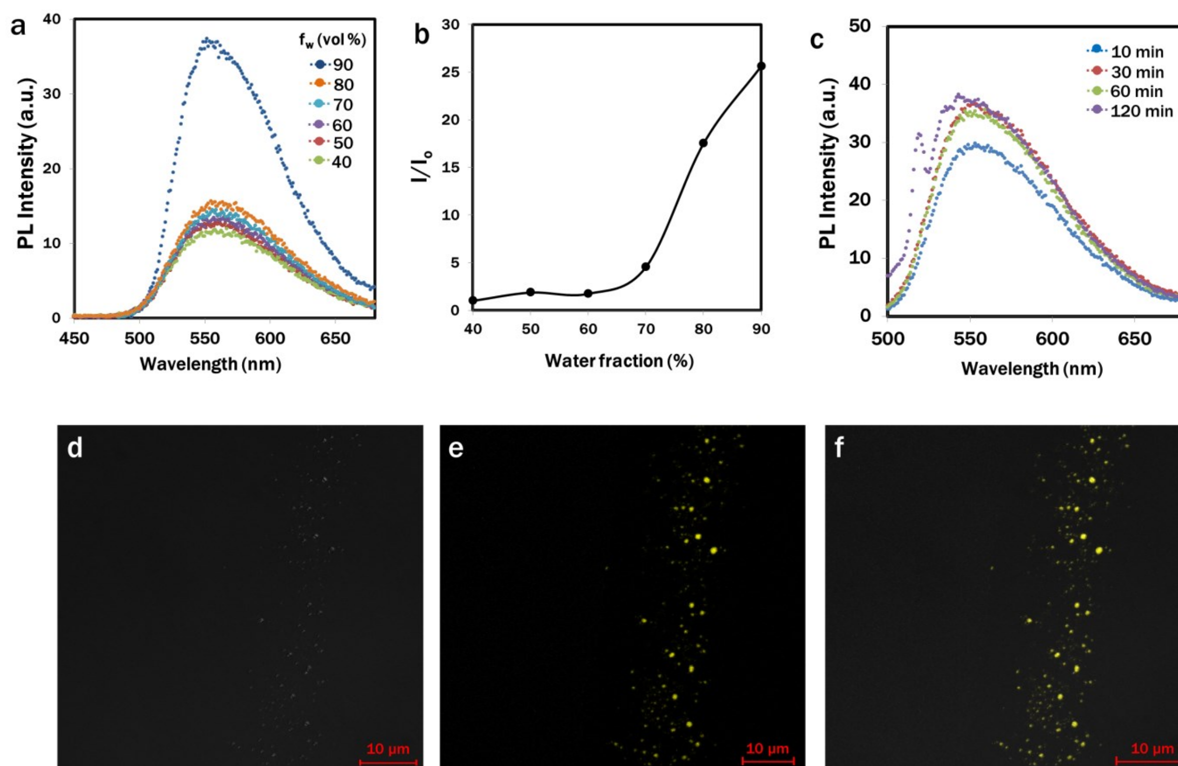
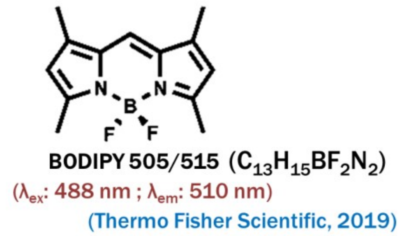
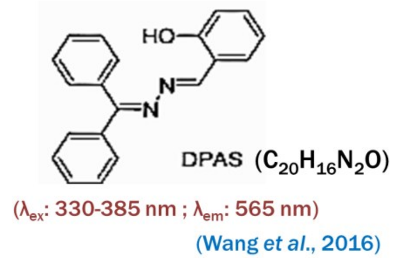
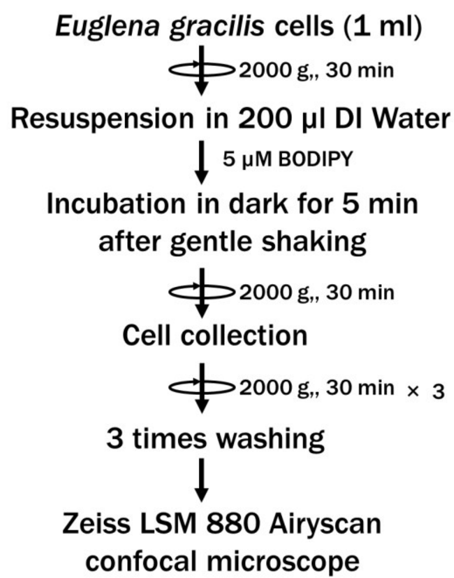


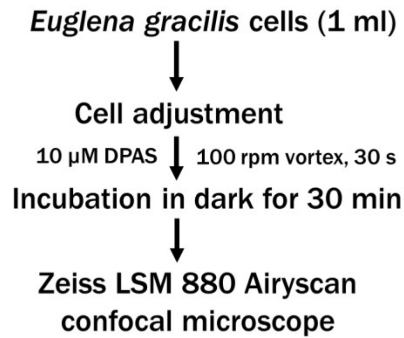
Fig. Fluorescence properties of DPAS. Fluorescence spectra of DPAS (10 μM) in DMSO-water mixtures (a-b); Fluorescence spectra of DPAS (10 μM) in *Euglena gracilis* cells at different time intervals (c); Fluorescence of DPAS in 2% sunflower oil under Zeiss LSM 880 Airyscan confocal microscope (d-f). Bright-field (d); aggregation of DPAS in sunflower oil (λ_{ex}: 405 nm, λ_{em}: 560 nm) (e); Merge (f).

Supplementary S4- Flow-diagram of sample preparation steps with BODIPY 505/515 and DPAS









Reference

S1. Y. Wu, J. Y. Qu, Autofluorescence spectroscopy of epithelial tissues, *J. Biomed. Opt.*, 2006, **11**, 054023.