Supplementary Information (SI) for Nanoscale.

Improve growth and lipid accumulation in microalgae with aggregation induced emission-based nanomaterial towards sustainable lipid production in microalgae

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Supporting information

1. Materials and Methods

1.1. Determination of the autofluorescence of *C. reinhardtii*

Different photosynthetic pigments in green microalgae exhibit strong autofluorescent properties upon excitation at appropriate wavelengths. Autofluorescence of chlorophyll, the primary photosynthetic pigment in *C. reinhardtii,* often causes interference with LDs during fluorescent staining. Before selecting a suitable AIE-based nanoprobe for lipid imaging, the autofluorescent spectrum of *C. reinhardtii* was determined with a fluorescent spectrophotometer to minimise the background noise. Cells were excited at 350, 405 and 488 nm. The emission peak at around 400 nm for the excitation of 350 nm was unclear (Fig. S1. a). However, two emission peaks at 470 and 685 nm for the excitation of 405 nm (Fig. S1. b) were supposed to be due to the number of redox ratios (NAD(P)H/FAD) and autofluorescence of chlorophyll[1,2]. However, maximum autofluorescence of chlorophyll was observed as a single peak when excited at 488 nm (Fig. S1. c).

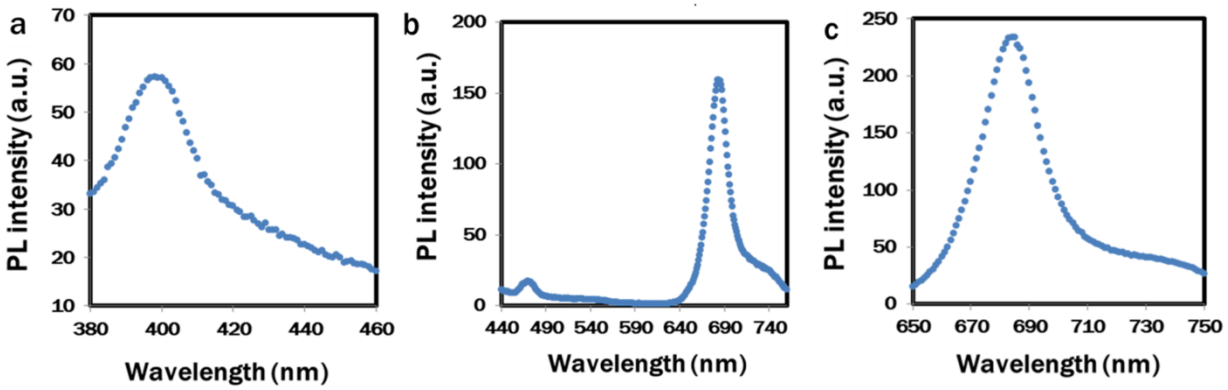

Fig. S1. Autofluorescence of *C. reinhardtii* excited at different wavelengths (a) λ_{ex}: 350 nm; (b)

λ_{ex}: 405 nm; (c) λ_{ex}: 488 nm

1.2. Determination of the lipid specificity of TPA-A

In the study, 10 µM of TPA-A was applied to 2% sunflower oil to identify the lipid specificity of TPA-A. Confocal microscopy revealed that TPA-A can stain the lipid molecules (Fig. S2). This lipid specificity of TPA-A is also supported by the previous study[3] where TPA-A was colocalised with a commercial lipid droplet probe, Nile Red.

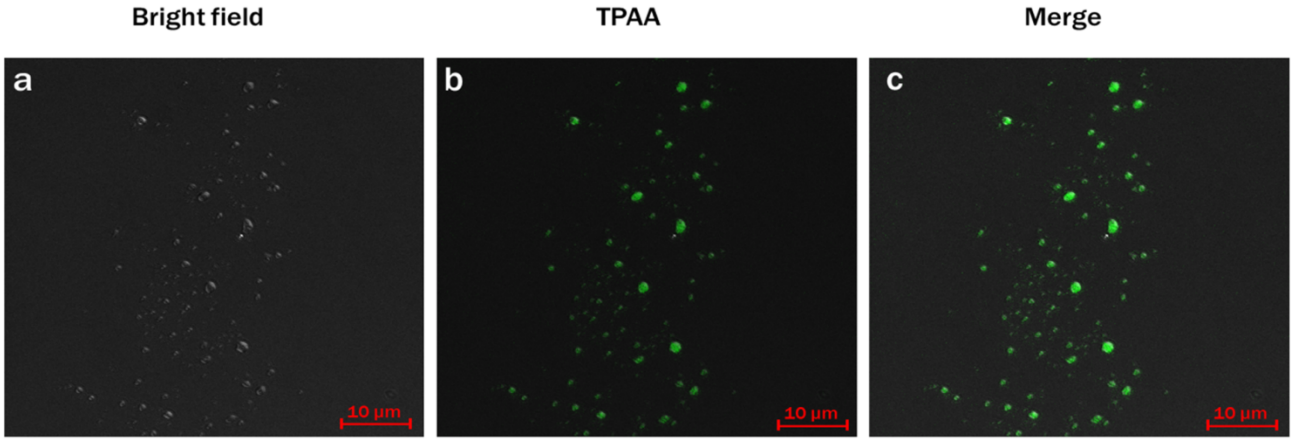

Fig. S2. Fluorescence of TPA-A in 2% sunflower oil under Zeiss LSM 880 Airyscan confocal microscope. (a) Brightfield; (b) TPA-A (λ_{ex}: 405 nm, λ_{em}: 410-597 nm) and (c) Merge.

1.3. GCMS analysis of TPA-A

To detect the residues of TPA-A, GCMS analysis was performed on day 7 with 10 µM TPA-A and compared with the initial day peak with the following conditions - Instrument: Waters Synapt HDMS, Capillary voltage: 2.25 kV, Ionisation mode: ESI positive, Mass range: 50-1000 m/z, Source Temp: 100 ^{0}C, Desolvation temp: 300 ^{0}C, Desolvation gas flow rate: 500 L/hr, Sampling

cone voltage: 20 V, Extraction cone voltage: 4 V. Samples were dissolved in DMSO-MeOH. At day 0, peaks were observed at the expected mass (Fig. S3), whereas no peaks occurred on day 7, Fig. S4 and Fig. S5). This might be due to the complete degradation of this molecule, which means no residues remain in the samples.

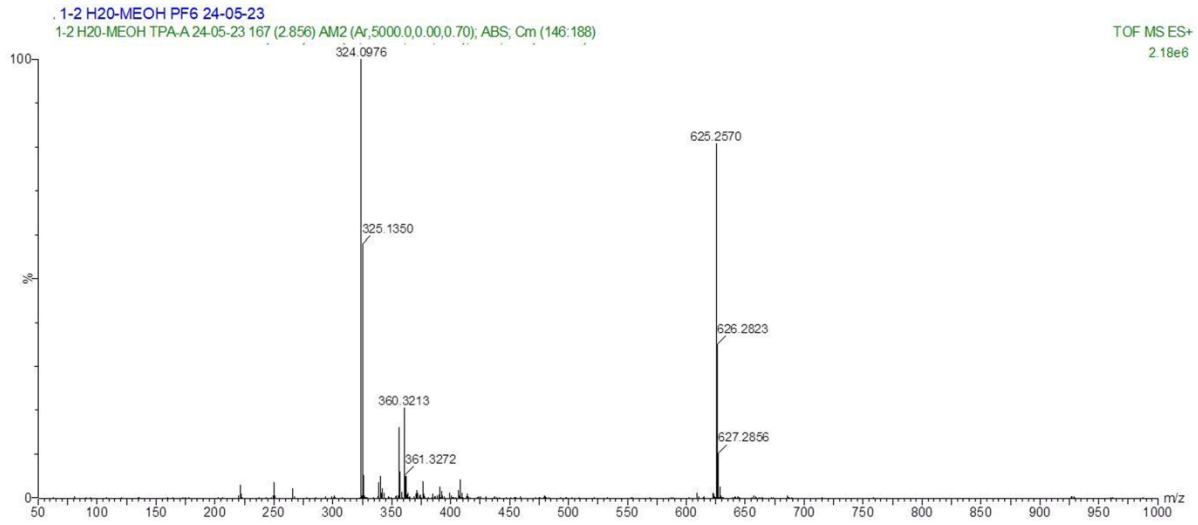

Fig. S3. GC-MS analysis of 10 µM TPA-A at day 0. Samples were prepared in DMSO-MeOH.

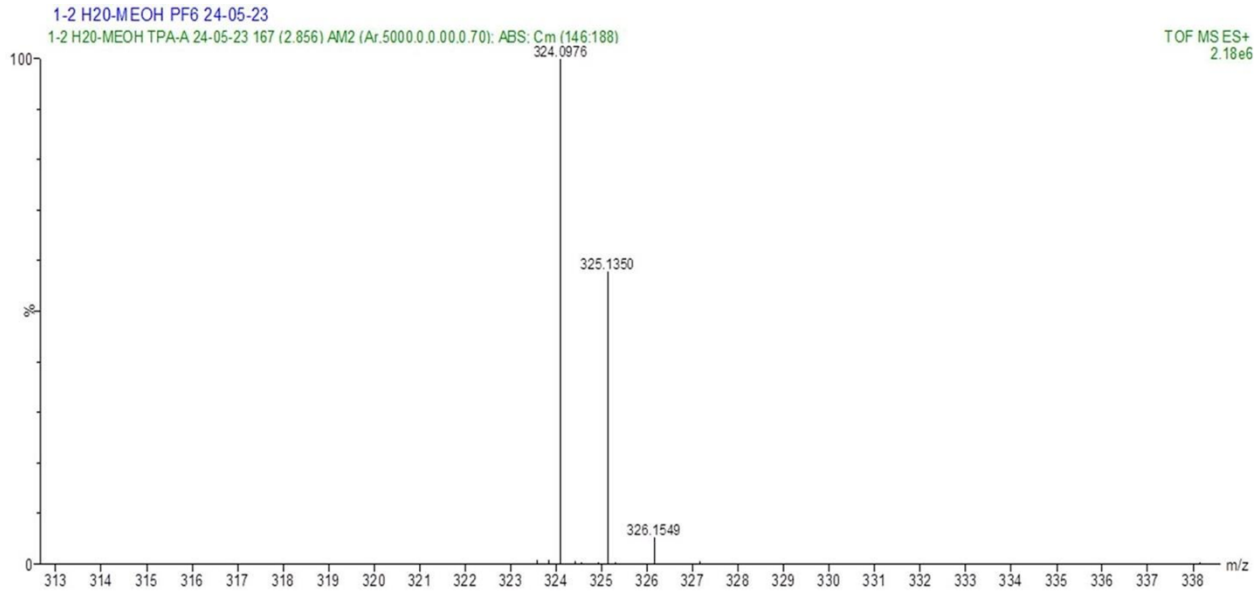

Fig. S4. GC-MS analysis of 10 μM TPA-A at day 7(wavelength 313-338 nm). Samples were prepared in DMSO-MeOH.

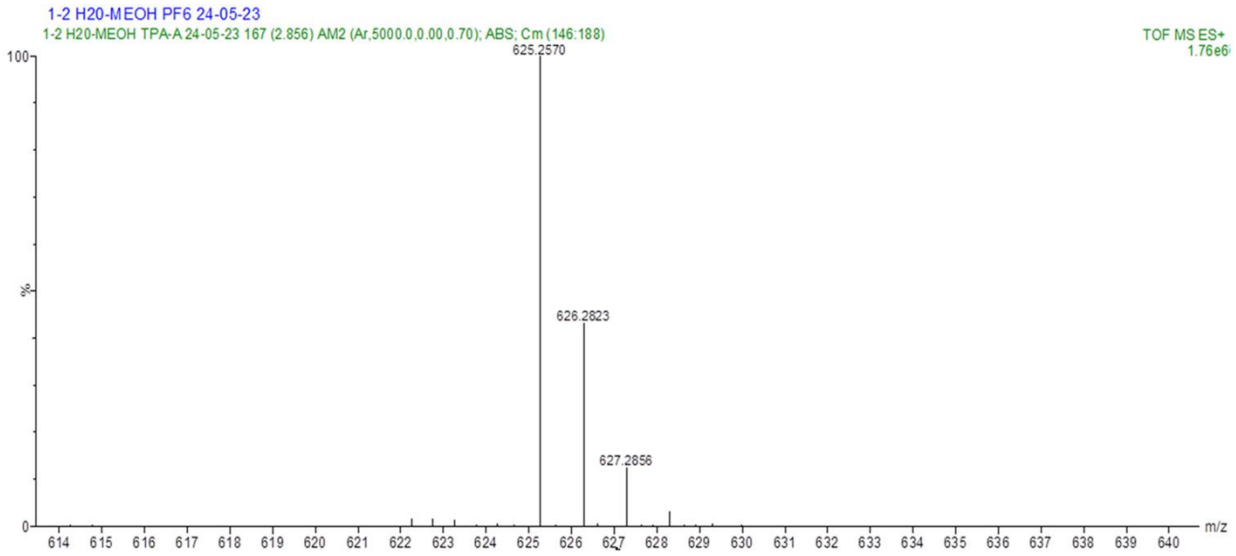

Fig. S5. GC-MS analysis of 10 μM TPA-A at day 7. Samples were prepared in DMSO-MeOH.

References

1 Y. Wu and J. Y. Qu, *J. Biomed. Opt*., 2006, **11**, 054023.

2 A. H. M. M. Reza, S. F. Rakhi, X. Zhu, Y. Tang and J. Qin, *Biosensors*, 2022, **12**, 208-232.

3 H. Liu, N. Yan, T. Y. Wong, H. Lam, J. W. Y. Lam, R. T. K. Kwok, J. Sun and B. Z. Tang, *ACS Nano*. 2022, **16**, 14973-14981.