SUPPORTING INFORMATION

Aggregation-induced emission fluorogen as biomarker to assess viability of microalgae in aquatic ecosystem

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Materials

All the reagents used in this study were purchased from Sigma-Aldrich unless otherwise specified. Trypsin was purchased from Invitrogen/Gibco (Cat. No. 15090-046). PBS was made according to the cold spring harbor laboratory protocol.[2] BSPOTPE was prepared according to published procedures.[3] Stock solution of BSPOTPE of a concentration of 5.0 mM was prepared by dissolving an appropriate amount of dye in Milli-Q lab water (Millipore, Billerica, MA). The solution was stored in dark before use.

Algae strain and culture conditions

Nannochloropsis oculata were obtained from the School of Biological Sciences, Flinders University. The cells were cultivated under continuous light with a light density of 85 μmol photons $m^{-2}s^{-1}$, continuously agitated by bubbling with sterile air, enriched with 0.5% CO_2 at 25 ± 1°C in a temperature-controlled room. The *N. oculata* cells were first diluted to 1:20 and then counted on a haemocytometer. Seawater was from filtered natural seawater with pH 8.4 and autoclaved before use.

BSPOTPE monitoring live and dead *N. oculata*

Steady-state fluorescence spectra were recorded on a Varian Cary Eclipse Fluorimeter (Varian Australia Pty Ltd). After live *N. oculata* were diluted to 25% of the original concentration with seawater, algal cells were incubated at different concentrations of BSPOTPE (0, 50, 100, 200 and 500 μM) for 2 min, and the PL was measured on the fluorimeter. In the meantime, live *N. oculata* were treated in 75% (v/v) ethanol for 30 min, and then incubated in BSPOTPE for 2 min, followed by photoluminescence (PL) assay (excitation wavelength 340 nm, emission wavelength 485 nm). Photographs of live and dead *N. oculata* stained with 200 and 500 μm of BSPOTPE were taken under illumination of a UV lamp (Spectronics, Westbury, NY, USA).

Monitoring PL intensity of live and dead *N. oculata* according to different time profiles

The PL intensities of live and dead *N. oculata* of 6×10^6/ml with 200 μM BSPOTPE were tested at different time points, i.e., 2, 30, 45 min, 1, 2, 3, 4.5, 6, 7.5, 9, 10.5 and 12 h,

respectively.

Measurement of *N. oculata* staining by BSPOTPE under the salinity control

As salinity may influence the staining process, we observed the PL of live *N. oculata* with BSPOTPE under the control of salinity. The fluorescence of a total of 500 μl *N. oculata* in seawater and 2 ml of PBS was monitored with 200 μM BSPOTPE. Salinity had negligible impact on the staining of *N. oculata* by BSPOTPE.

Evaluating PL intensity of BSPOTPE with different concentration of *N. oculata* in the PBS buffer

We made a serial dilution of *N. oculata* by the PBS buffer. Then, the PL intensity was measured with the presence of 200 μM BSPOTPE. The *N. oculata* concentration varied from 0.05 $\times 10^6$/ml to 20×10^6/ml and the incubation time was 2 min.

Live and dead *N. oculata* staining with BSPOTPE or propodium iodine

The *N. oculata* was treated with 75% ethanol for 30 min, which can effectively kill the algae, and incubated with BSPOTPE (200 μM) or propodium iodine (PI, 50 μM) for 20 min. Then the cells were loaded onto a microscope slide and covered with a cover slip and imaged by an Olympus AX70 fluorescence imaging microscope (Tokyo, Japan) equipped with a charge-coupled device (CCD) camera (Cool-SNAP; Roper Scientific, San Diego) (excitation wavelength: 340 nm; PI: 510 nm; 100× magnification). A control was done by replacing ethanol with seawater and stained with BSPOTPE/PI. The final concentration of live or dead *N. oculata* was 7.5×10^6/ml.

Proposed staining mechanism of *N. oculata* by BSPOTPE

The *N. oculata* was treated with trypsin to digest the membrane proteins and leave the intact cell walls.[5] Alternatively, proteinase K, regarded bearing stronger protein cleavage ability was applied to treat live *N. oculata* to break up cell membrane. Firstly 1% trypsin and 0.5% proteinase K were measured for PL intensity with 200 μM BSPOTPE separately. After 1% trypsin was mixed with 3.36×10^6/ml *N. oculata* for 2 h, 200 μM BSPOTPE was added and PL intensity was monitored. For the 0.5% proteinase treatment, the same procedure was

applied and the *N. oculata* concentration was 6×10^6/ml.

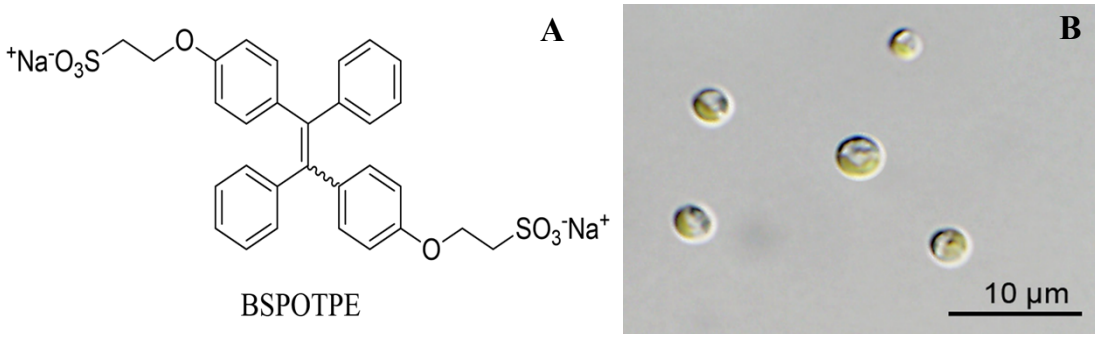

Figure S1. (A) Molecular structure of 1, 2-bis[4-(3-sulfonatopropoxyl)phenyl]- 1,2-diphenylethene sodium salt (BSPOTPE); (B) *Nannochloropsis oculata*, courtesy and adapted from Dr Gert Hansen (Scandinavian Culture Collection of Algae & Protozoa, Department of Biology, University of Copenhagen) [1]

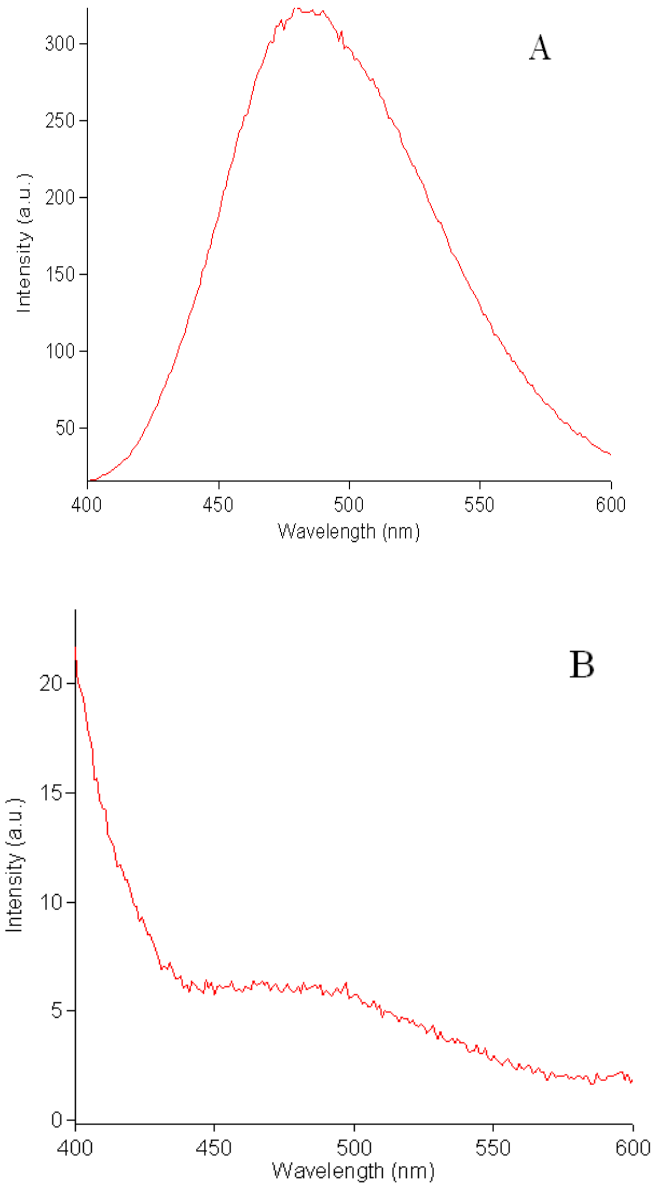

Figure S2. Emission spectra of BSPOTPE (200 μM) with (A) live *N. oculata* and (B) dead N. oculata, both at the concentration of 10×10^6/ml. λ_{ex}= 340 nm, λ_{em}= 400-600 nm, incubation time: 2 min

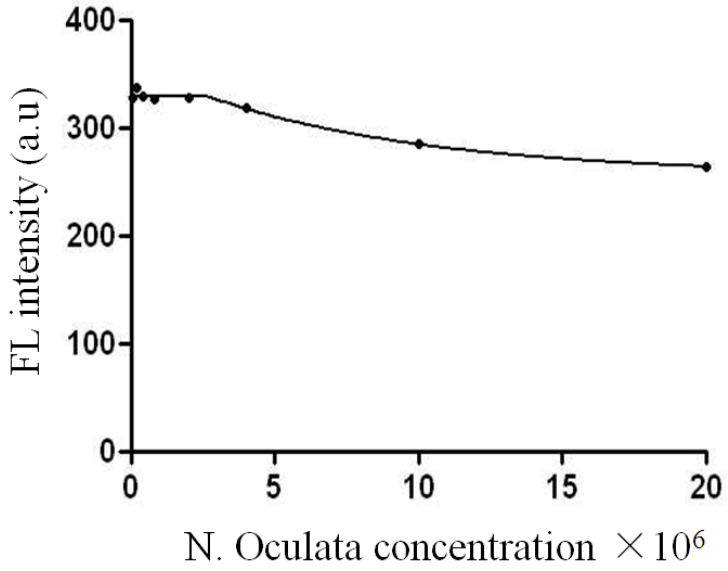

Figure S3. FL intensity of different concentration of live *N. oculata* with BSPOTPE (200 μM) under the control of salinity with salinity at about 13.8‰. λ_{ex}= 340 nm, λ_{em}= 400-600 nm, and incubation time: 2 min

Reference

1. http://www.sccap.dk/pix/K-1281.jpg
2. http://cshprotocols.cshlp.org/content/2006/1/pdb.rec8247.full?text_only=true
3. Tong H, Hong Y, Dong Y, Häussler M, Li Z, Lam JW, Dong Y, Sung HH, Williams ID, Tang BZ. Protein detection and quantitation by tetraphenylethene-based fluorescent probes with aggregation-induced emission characteristics. J Phys Chem B 2007, 111 (40): 11817-23.
4. Hayden SC, Zhao G, Saha K, Phillips RL, Li X, Miranda OR, Rotello VM, El-Sayed MA, Schmidt-Krey I, Bunz UH. Aggregation and interaction of cationic nanoparticles on bacterial surfaces. J Am Chem Soc. 2012, 134 (16): 6920-3.