Electronic Supplementary Information (ESI)

Spatiotemporal monitoring of intracellular metabolic dynamics by resonance Raman microscopy with isotope labeling

Yusuke Yonamine,*[a] Kotaro Hiramatsu,[b,c,d] Takuro Ideguchi,[c,d,e] Takuro Ito,[f] Tomomi Fujiwara,[g] Yoshiko Miura,[g] Keisuke Goda,[b,f,h,i] and Yu Hoshino*[g]

[a] *Research Institute for Electronic Science, Hokkaido University, Kita 21, Nishi 10, Kita-ku, Sapporo 001-0021, Japan.*
[b] *Department of Chemistry, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.*
[c] *Research Centre for Spectrochemistry, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.*
[d] *PRESTO, Japan Science and Technology Agency, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.*
[e] *Institute for Photon Science and Technology, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.*
[f] *Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi-shi, Saitama 332-0012, Japan.*
[g] *Department of Chemical Engineering, Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan.*
[h] *Institute of Technological Sciences, Wuhan University, Hubei 430072, China.*
[i] *Department of Bioengineering, University of California, Los Angeles, California 90095, USA.*

E-mail: yonamine@poly.es.hokudai.ac.jp, yhoshino@chem-eng.kyushu-u.ac.jp

Experimental

Algal cells and culture conditions

Haematococcus lacustris NIES-144 was provided by the Microbial Culture Collection at the National Institute for Environmental Studies (NIES, Tsukuba, Ibaraki, Japan). The autotrophic medium AF-6 was used for culturing (working volume: 20 mL). Cells were grown in culture flasks (polystyrene suspension culture flask with filter caps, 50 mL volume, Greiner Bio-One) in static conditions under 14h:10h light:dark cycle illumination (approximately 50 μmol/m^2 s^{-1}) at 28 °C. For AXT induction, *H. lacustris* cells were grown in normal AF-6 medium at pH 6.6 for 5 days for pre-culture. During the exponential growth phase, cells in the pre-culture were transferred to nitrogen-deficient AF-6 media (without NH_4NO_3 and $NaNO_3$) in culture flasks (polystyrene suspension culture flask with standard screw cap, 250 mL volume, Greiner Bio-One) at a cell density of 1×10^5 cells mL^{-1} (working volume: 20 mL). After purging air with filtered (0.22 μm) nitrogen gas and enclosing 1 vol% CO_2 filtered gas (details are described below) in the culture flask, cells were incubated under continuous light illumination (approximately 150 μmol/m^2 s^{-1}) at 28 °C. For resonance Raman microscopic analysis, cells were immobilised with 0.25% glutaraldehyde solution and concentrated by centrifugation (1100 × *g* for 5 min).

Preparation of mixtures of $^{13}CO_2$ and $^{12}CO_2$ gas

The mixtures of $^{13}CO_2$ and $^{12}CO_2$ gases were prepared from a neutralization reaction of $NaHCO_3$ with HCl. Aqueous solutions of 0.5 M $NaH^{13}CO_3$ (^{13}C: 99%, Cambridge Isotope Laboratories, Inc) and 0.5 M $NaH^{12}CO_3$ (Nakarai Tesque, Inc.) were mixed in various ratios (total 2 mL): the molar ratios of $NaH^{13}CO_3$ to $NaH^{12}CO_3$ were 0:100, 25:75, 50:50, 75:25, and 100:0. To the mixture of ^{13}C- and ^{12}C-$NaHCO_3$ solution (2 mL) in a 50 mL syringe, 1M HCl aqueous solution (1.2 mL) was added to generate ^{13}C- and ^{12}C-CO_2 mixed gas. The collected gas (5 mL) in a syringe was mixed with 495 mL of air (atmospheric $^{12}CO_2$ concentration: approximately 0.04 vol%) to prepare a 1 vol% mixture CO_2 gas of $^{13}CO_2$ and $^{12}CO_2$ with desired ratios.

Switching atmosphere from $^{13}CO_2$ to $^{12}CO_2$

1 vol% $^{13}CO_2$ and $^{12}CO_2$ gases were each prepared by mixing 5 mL of $^{13}CO_2$ gas (^{13}C: 99%, Cambridge Isotope Laboratories, Inc) and $^{12}CO_2$ gas, respectively, with 495 mL of air (atmospheric $^{12}CO_2$ concentration: approximately 0.04 vol%). The incubational atmosphere was switched from $^{13}CO_2$ to $^{12}CO_2$ by first removing $^{13}CO_2$ by purging with filtered nitrogen gas and then passing 1 vol% $^{12}CO_2$ filtered gas through the culture media (total 500 mL). Filtered 1 vol% $^{12}CO_2$ gas was enclosed in the culture flask for further incubation. The incubation atmosphere was switched from $^{12}CO_2$ to $^{13}CO_2$ with a procedure analogous to that described above.

Resonance Raman microscopy of ^{13}C-incorporated *H. lacustris* cells

Resonance Raman spectra were acquired using a confocal Raman microscope (LabRAM Aramis, Horiba Scientific, Japan) equipped with an integrated Olympus microscope. A 50x dry objective was used to acquire Raman signals from single *H. lacustris* cells. Laser light was targeted onto the cell using an integrated color camera and a motorized XYZ stage. Raman scattering was excited with a 532-nm laser (ventus 532, Laser Quantum) with 1/10 or 1/100 laser filters. Each Raman spectrum was acquired in the range between 500 and 2000 cm^{-1}. The system was run with a confocal pinhole diameter of 100 μm and a diffraction grating with a groove density of 600 grooves/mm. For resonance Raman imaging, SWIFT imaging function mode was used, which significantly reduces the time needed to generate Raman images. A 20x dry objective was used to acquire low-magnification Raman images of concentrated *H. lacustris* cells. 125 × 100 pixels were acquired for one frame (500 μm × 400 μm) with a pixel dwell time of 0.1 s. A 50x dry objective was used to acquire magnified Raman images of single *H. lacustris* cells. 50 × 50 pixels were acquired for one frame (50 μm × 50 μm) with a pixel

dwell time of 0.1 s. Raman images were generated by classical least squares modeling of ^{13}C- and ^{12}C-AXT spectra as spectral bases. At each image pixel position, a linear combination of factors was calculated to estimate the measured spectrum. ^{13}C- and ^{12}C-AXT in the images were color-coded as red (^{13}C-) and green (^{12}C-), respectively, with a software provide by the instrument manufacturer (LabSpec, Horiba Scientific, Japan).

Peak resolution of resonance Raman spectra

In the atmosphere switching experiments, the Raman spectra obtained from multiple *H. lacustris cells* were decomposed into two basis spectra using a Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) algorithm.[1–4] First, a series of spectra (n=150; 30 spectra for each condition) was analyzed by singular value decomposition (SVD), which gives two singular values significantly higher than the noise level. While we can know the number of basis spectra required to reproduce all of the experimentally obtained spectra in SVD analysis, the eigenspectra obtained in SVD cannot be directly attributed to the chemical composition of the cell, as these have negative peaks. In order to obtain basis spectra that are chemically meaningful, the series of spectra were resolved into superpositions of two non-negative spectra with non-negative weight coefficients by the ALS optimization.[1–4] The obtained basis spectra are consistent with the spectra of *H. lacustris* cells cultured under pure $^{12}CO_2$ or $^{13}CO_2$. Thus, the ratios of the weight coefficients are interpreted as the relative concentration of ^{13}C-AXT to total AXT in *H. lacustris* cells. The ratios were calculated for each cell and plotted in Fig. 4d. In the pulse incubation experiments, series of spectra were reproduced by the basis spectra obtained in the above analysis of the data from the switching experiments. The ratios of the weight coefficients in the fitting analysis are interpreted as the relative concentration of ^{13}C-AXT to total AXT in *H. lacustris* cells and shown in Fig. 5b.

Supporting figures

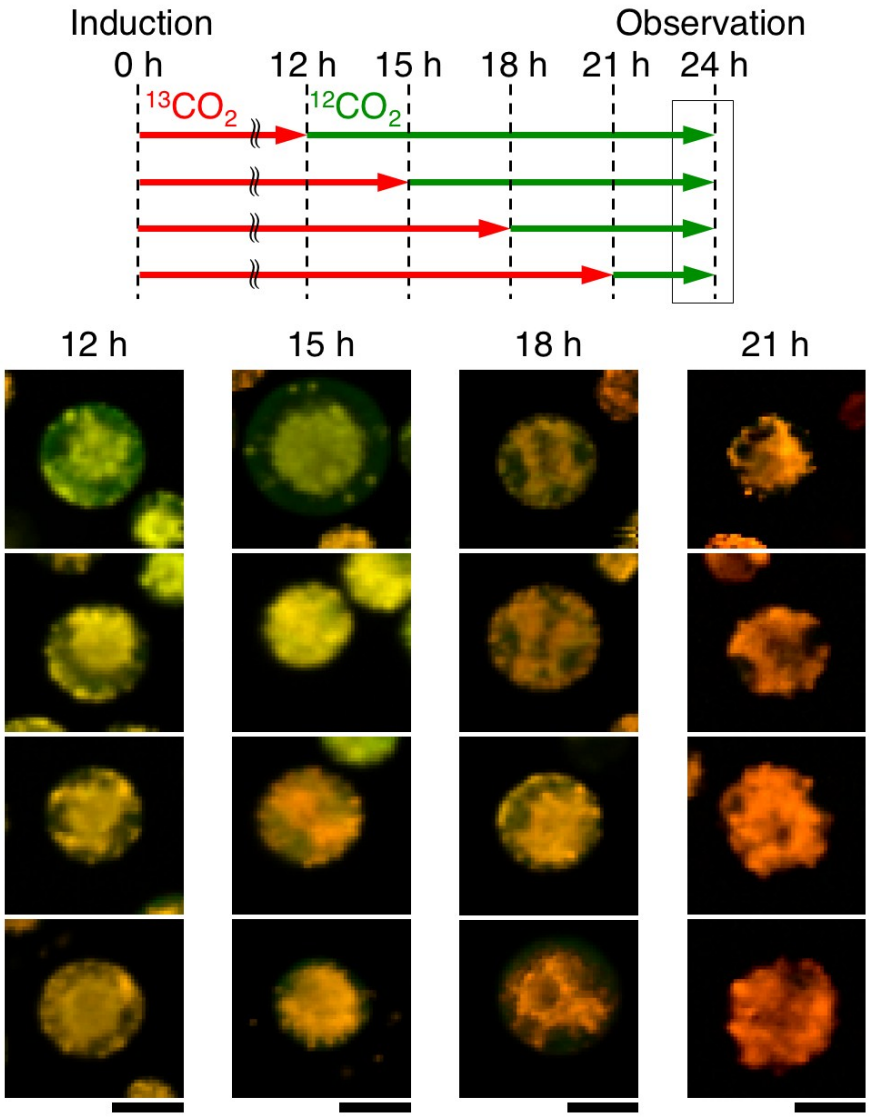

Fig. S1 Resonance Raman images of individual *H. lacustris* cells incubated under the conditions shown in Figure 4a. ^{13}C- and ^{12}C-AXT are color-coded in red and green, respectively. Scale bars: 20 μm.

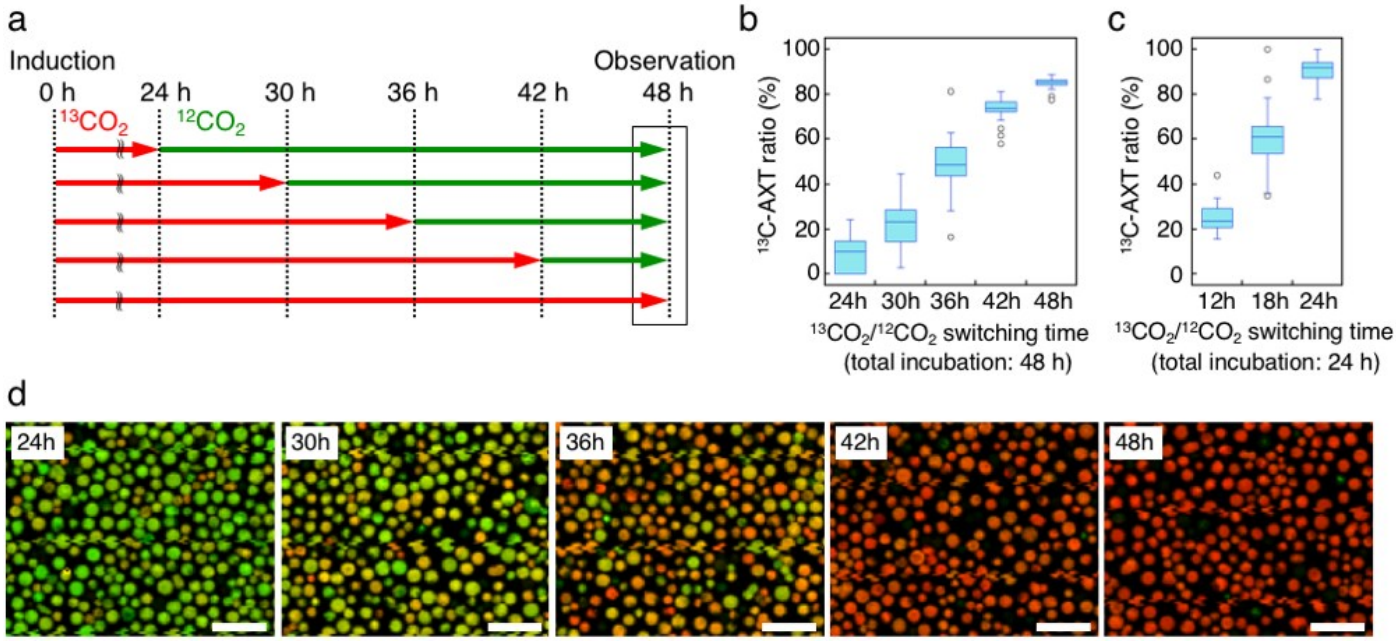

Fig. S2 Resonance Raman imaging of *H. lacustris* cells incubated with atmosphere switching from $^{13}CO_2$ to $^{12}CO_2$ at a 6-hour interval with a total AXT-induction of 48 h. (a) Incubation timeline of *H. lacustris* cells with incubation switching from $^{13}CO_2$ to $^{12}CO_2$ at various timings within 48 h. (b) Box-and-whisker plot of ^{13}C-AXT ratios in resonance Raman spectra of *H. lacustris* cells (n = 30), where switching times correspond to the timelines in (a). (c) Box-and-whisker plot from Figure 4d showing the data from the 12 h, 18 h, and 24 h conditions. (d) Low-magnification resonance Raman images of *H. lacustris* cells incubated under the conditions shown in (a). ^{13}C- and ^{12}C-AXT are color-coded with red (^{13}C) and green (^{12}C), respectively. Scale bars: 100 μm.

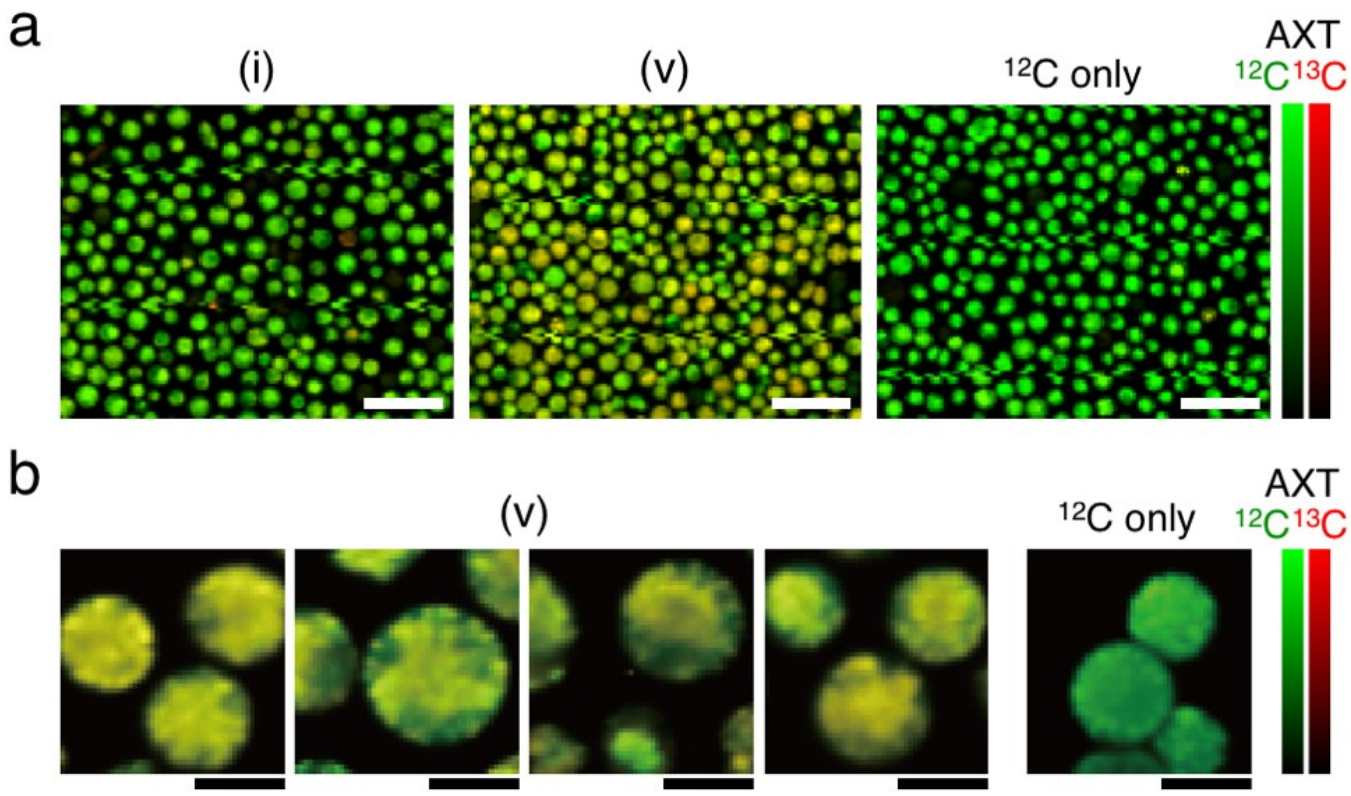

Fig. S3 Resonance Raman images of *H. lacustris* cells incubated under the conditions shown in Figure 5a. ^{13}C- and ^{12}C-AXT are color-coded in red (^{13}C) and green (^{12}C), respectively. (a) Low-magnification resonance Raman images. Scale bars: 100 µm. (b) High-magnification resonance Raman images of condition (v) and ^{12}C only. Scale bars: 20 µm. Color bars: Intensities of Raman spectra of ^{13}C- (red) and ^{12}C-AXT (green).

References

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