Micropatterning of Living Cyanobacteria on Gold Nanostructures based on Localized Surface Plasmon Excitation

Shota Naka[1], Tatsuya Shoji[1], Yumi Wakisaka[2], Kei Murakoshi[2], Tadashi Mizoguchi[3], Hitoshi Tamiaki[3], Yasuyuki Tsuboi[1]*

[1]Graduate School of Science, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558-8585, Japan

[2]Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo 060-0808, Japan

[3]Graduate School of Life Science, Ritsumeikan University, Kusatsu, Shiga, 525-8577, Japan

**E-mail: twoboys@sci.osaka-cu.ac.jp*

Fixation of photosynthetic bacteria on a plasmonic substrate is essential methods to realize plasmon-enhanced light harvesting. For this purpose, it is necessary to fix photosynthetic bacteria at metallic nanostructures. One of the powerful approaches to it is plasmonic optical tweezers (POT). POT is an optical manipulation technique based on localized surface plasmon (LSP).[2] So far, using POT, we have trapped quantum dots, DNA, and artificial polymer chains.[2,3,4] In this study, we investigated a novel method to fix living cyanobacteria on a metallic nanostructure.

We used spherical cyanobacteria with the diameter of ca. 2 μm (*Synechocystis* sp. PCC *Δslr1923*) dispersed in water. These cyanobacteria have fluorescent photosynthetic pigments. To evaluate the viability of cyanobacteria after LSP excitation, they were stained with fluorescent dye molecules of a viability test kit. As a plasmonic nanostructure, we fabricated gold nanopyramidal nanodimer arrays by angular-resolved nanosphere lithography.[5] The substrate had a broad extinction band around 800 nm corresponding to gap-mode LSP. We introduced a near-infrared laser beam as plasmon excitation light, and a visible laser beam or a mercury lamp as fluorescence excitation light into an inverted microscope, with which we observed fixation behaviors.

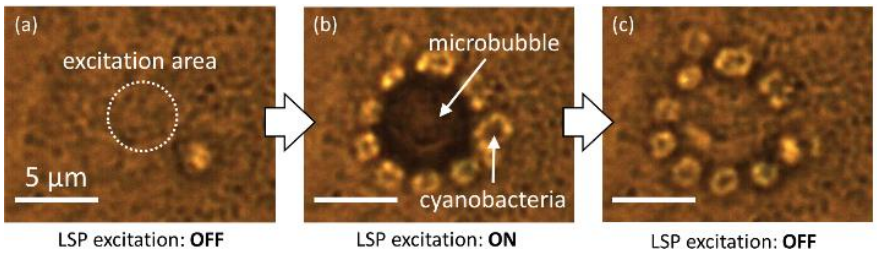

Figure. 1 Bright field images of cyanobacteria micro-pattern a) before turning on LSP excitation, b) upon the excitation, c) after the stop of the excitation.

Upon intense LSP excitation (100 kW/cm^2), a microbubble appeared at the excitation area. Subsequently, cyanobacteria were assembled around the microbubble (Fig. 1b). Just upon the stop of the excitation, the microbubble disappeared. However, the cyanobacteria still remained at the same position (Fig. 1c). These results indicated that the cyanobacteria were fixed on the plasmonic substrate through the microbubble formation. Increasing the excitation light intensity for LSP, the number of the fixed cyanobacteria also increased. The assembling behavior would be attributed to Marangoni flows and capillary flows. Along these flows, cyanobacteria were attracted to the

interface between the microbubble and the substrate.

We measured fluorescence spectra from the fixed cyanobacteria. It was revealed that photosynthetic pigments were not decomposed after the fixation (Fig. 2a). Moreover, we observed cyanobacteria stained with the dye molecules. The fluorescence images indicated that the fixed cyanobacteria were partly alive (Fig. 2b, c).

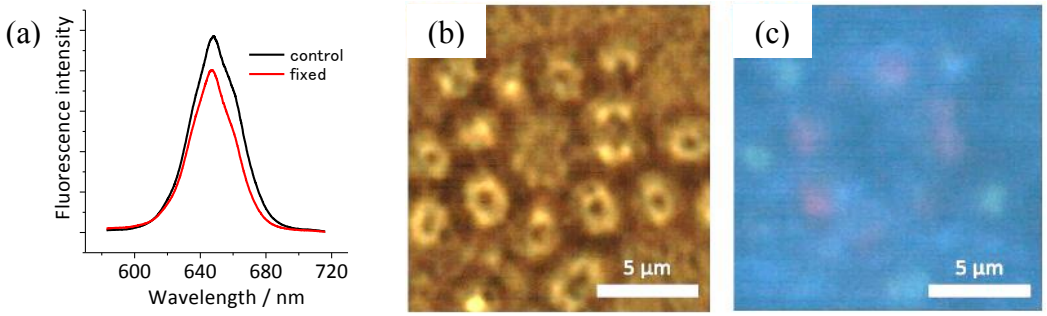

Figure. 2 a) Fluorescence spectra of cyanobacteria. b) A bright field image and c) a fluorescence image of fixed cyanobacteria.

In conclusion, we succeeded in micro-patterns of living cyanobacteria on gold nanostructures by plasmon-induced microbubble formation. Upon intense LSP excitation, a microbubble was transiently produced, leading to assembling of living cyanobacteria. By changing the excitation light intensity for LSP, we controlled the number of cyanobacteria fixed on the structures. The viability evaluation indicated that the fixed cyanobacteria were alive.

Acknowledgements: This work was supported by JSPS KAKENHI Grant Numbers JP26288011, JP16K17922, JP16H06506/JP16H06507 in Scientific Research on Innovative Areas "Nano-Material Manipulation and Structural Order Control with Optical Forces".

References:

[1] A. Govorov and I. Carmeli, *Nano Lett.*, **2007**, 7, 620
[2] Y. Tsuboi, H. Ishihara *et al.*, *J. Phys. Chem. Lett.*, **2010**, 1, 2327
[3] T. Shoji, Y. Tsuboi *et al.*, *J. Phys. Chem. C*, **2013** 135, 6643
[4] M. Toshimitsu, Y. Tsuboi *et al.*, *J. Phys. Chem. C*, **2012**, 116, 14610
[5] M. Takase, K. Murakoshi *et al.*, *Nat. Photon*, **2013**, 7, 550