

A Photocaged DNA Nanocapsule for Controlled Unlocking and Opening inside the Cell

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ABSTRACT: We report a nano-sized DNA capsule with a photo-inducible mechanical unlocking system for creation of a carrier for delivery system to the cells. Photocage system was introduced into the nanocapsule (NC) for control of opening of the NC with photoirradiation. The open of the NC was observed by atomic force microscopy (AFM), and the dynamic opening of the NC was examined by fluorescence recovery from the quenching. The photocaged NC was introduced to the cell without toxicity and observed in the cytoplasm, and the photo-induced open of the NC was observed in the cell. The selective unlocking and opening of the caged-NC in a single cell was successfully achieved by a laser irradiation to individual cells.

Development of a nano-sized carrier for the cellular incorporation is one of the important research topics in nanobiotechnology. Because of their potential applications for imaging, diagnosis, and therapeutics, nano-sized signal- and stimuli-responsive carriers are attracting attention.¹ Especially, the designable nano-sized structures have been realized by the progress of the DNA origami technology, which enables the reliable construction of various nanostructures.²⁻⁴ Advantages for the use of DNA is feasible introduction of functions using DNA oligonucleotides and many researchers reported the incorporation of functionalities into DNA nanostructures.⁴ These DNA origami structures have been used for targeting to introduce into cells for the therapeutic purposes.⁵⁻⁷ In addition, regulating configurational changes of three-dimensional (3D) structures using a dynamic system is emerging,¹¹⁻¹⁵ and some of them have been applied for control of biological activities.^{7,16} For initiating the reaction and subsequent conformational change of the nanostructures, photochemical reactions are advantageous over other molecular switching reactions because the reaction can be initiated and activated by controlled photoirradiation and it is no need to add necessary molecules outside.¹⁷⁻¹⁸ We have constructed photoresponsive 2D and 3D DNA origami nanostructures to be employed to control assembly/disassembly and conformational changes by photoirradiation.¹⁹⁻²¹

Herein, we intended to incorporate photoresponsive DNA nanostructure to the cell and manipulate its conformational change inside the cell. We prepared a square bipyramidal DNA nanocapsule (NC) equipped with a photoresponsive lock/unlock system.¹⁹ As illustrated in Figure 1a, photocleavable (PC) DNA strands were incorporated to the NC to lock the closed form. By photoirradiation,

cleavage of the PC-strands could unlock and induce open of the NC. Therefore, the caged-NC incorporated into cells should be unlocked and subsequently opened inside the cell by photoirradiation.

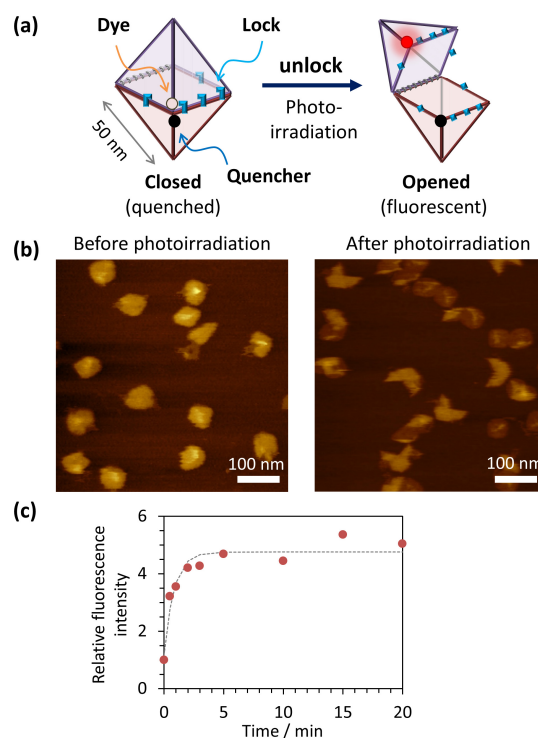


Figure 1. Photocaged DNA nanocapsule (caged-NC) with a lock/unlock system. (a) Schematic representation of the caged-NC that changes the conformation from a closed to open form by photoirradiation. Fluorescence dye and quencher are attached to the front corner of the caged-NC.

(b) AFM images of the caged-NC (left) and UV-irradiated caged-NC (right). (c) Time dependent uncaging of the NC with UV-irradiation. Unlocking and opening of the caged-NC was monitored by fluorescence recovery from the quenched state.

First, we prepared the DNA nanocapsule (NC) in closed forms using PC-strands. The NC was assembled in a solution containing M13mp18 and staple strands in a buffer containing 20 mM Tris buffer (pH 7.6), 10 mM MgCl₂ and 1 mM EDTA. First, the open structure was formed by annealing from 85 °C to 15 °C at a rate of -1.0 °C/min, then PC-strands were added and annealed from 35 °C to 15 °C at a rate of -0.5 °C/min to lock the structure. The assembled caged-NC was confirmed by gel electrophoresis and AFM images (Figures 1 and S2). Then five PC-strands were incorporated into the front and side edges to connect top and bottom pyramids by annealing (Figure S1). For the preparation of the caged-NC, the PC linkers were incorporated to the specific staple strands (Table S1). We selected these positions to keep the robustness of the NC when closed, which was examined in the previous study.¹⁹ In the gel electrophoresis, the caged-NC migrated faster than opened NC (Figure S2). In the AFM image, the caged-NC was observed as a double-layered square shape, in which a square bipyramidal structure was compressed on the mica surface, and the observed structures were similar to the closed NC (Figures 1b).¹⁹ This indicates that the PC-strands tightly connect the top and bottom pyramids. Then, the closed caged-NC was treated with UV irradiation for 10 min, and fully opened structures were observed as a double-layered half-opened structure and a fully opened single-layered structure with two connected squares (Figures 1b).

Photo-induced opening of the caged-NC was examined in solution. In the gel electrophoresis, the caged-NC with UV-irradiation migrated slower than the initial caged-NC depending on the irradiation time (Figure S2). Next, for observing the dynamic opening of the caged-NC in solution, the fluorescent dye and quencher was attached to the front corner of the top and bottom of the square bipyramid, respectively (Figures 1a and S1). When the caged-NC is unlocked and open with UV-irradiation (350 nm), the fluorescence quenching should be recovered. The caged-NC was labelled with Cy3 dye and BHQ-2 quencher at the front corners of pyramid (Figure 1a and S1). After the photoirradiation, the increase of the fluorescence was observed by cleaving the PC-strands, showing that the unlocking of the closed caged-NC occurred. The intensity of fluorescence was time-dependent and the unlocking completed within 5 min irradiation. This results shows that the unlocking of the caged-NC could be easily monitored using fluorescence quenching and its recovery.

Then the caged-NC with fluorescent dyes was incorporated into the cell by incubating the NC with cell. Normal Human Epidermal Keratinocytes (NHEK) was used for the experiment. The caged NC solution (40 nM) was added to the medium and the mixture was incubated at 37 °C for 1 h. After the incubation, the cellular uptake of FAM-

labelled caged-NC was observed (Figure 2a). The FAM fluorescence was widely spread in the cytoplasm. To examine cytotoxicity, LDH assay and MTT assay were performed (Figure 2b). By changing the concentration of the caged-NC, no significant cytotoxicity was observed up to the concentration of 8 nM that was employed in the following experiments.

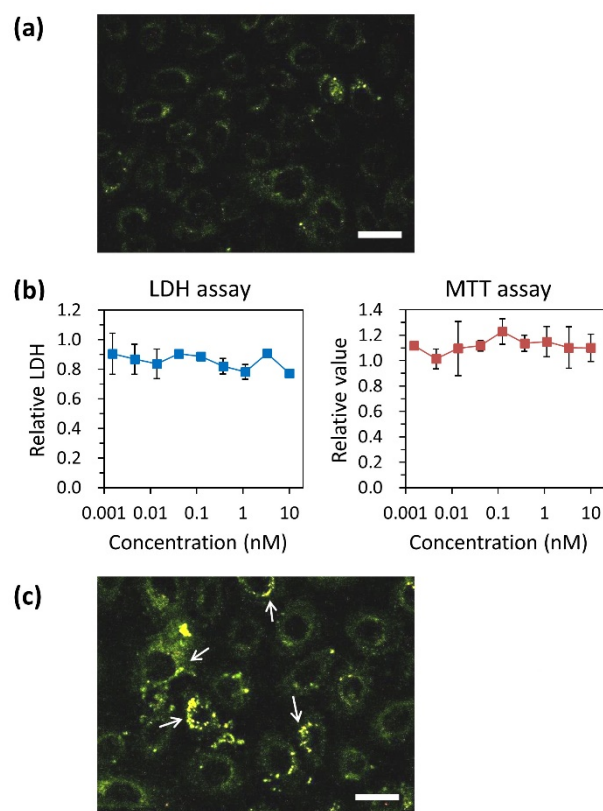


Figure 2. Cellular uptake of caged-NC. (a) Confocal laser scanning microscope (CLMS) image of the cells after incubation with FAM-labelled caged-NC with Cy3 dye/BHQ-2 quencher. (b) Cytotoxicity LDH assay and MTT assay. Each assay was independently repeated three times. Error bars represent S.D. (c) CLMS image of the cells after photoirradiation to the incubated cell with FAM-labelled caged-NC with Cy3 dye/BHQ-2 quencher. Scale bar 20 μ m.

We next examined the unlocking of the caged-NC in the cell. The photoirradiation was performed after the caged-NC was incorporated to the cell. After photoirradiation (bandpass filter with 330-385 nm) for 40 s, the fluorescence of the Cy3 dye increased in the cell (Figure 2c and S3). This indicates that the fluorescence of the quenched Cy3 was recovered by unlocking the caged-NC with photoirradiation. After photoirradiation, increased fluorescence was observed widely in the cytoplasm and partially colocalized to organelles in cell (arrows in Figure 2c). The result shows that the unlocking and subsequent opening of the NC occurred in the cell, and the open of the caged-NC in the cell was controlled with photoirradiation.

We next performed the single cell photoirradiation for the selective opening of the caged-NC in the individual cells (Figure 3a). The caged-NC was labelled with Alexa 488 dye and the opening of the NC was monitored by quenching and recovery of the fluorescence of the Alexa 647 dye/BHQ-3 quencher pair attached to the front corner of the NC (Figure S1). The single cell photoirradiation was performed using a laser with 405 nm wavelength. The obtained CLMS images were analyzed using ratio images (Alexa 647 (red)/Alexa 488 (green) channel). Three cells that the caged-NC was uptaken were selected and individually irradiated (yellow circled cells 1-3 in Figure 3b). Before the laser irradiation (Figure 3b left), the cells showed modest red color (Alexa 647 intensity). After irradiation, significant increase of the red color area in the cells (Figure 3b right). For fully open the NC, five PC-

strands should completely react to be cleaved, so that the color change was attributed to the opening of the NC. We obtained time-lapsed images every 5 s to observe the state of the caged-NC in the cell in detail. The time-lapsed single cell images were obtained and shown in Figure 3c and 3d. The laser irradiation to the cell was performed for 40 s. In the Figure 3c, the color change of the cell (cell 1) in the images was modest before the laser irradiation. After the irradiation (image 4), the fluorescence intensity attributed to the unlocking and opening of the caged-NC significantly increased and remained after turning off the irradiation. We obtained successive images showing the changes of the fluorescence intensity in the single cells by irradiating the laser individually (Figure 3d). After the irradiation to the cell (cell 2; left side), conformational change of the caged-NC to be opened occurred (image 4). Fur-

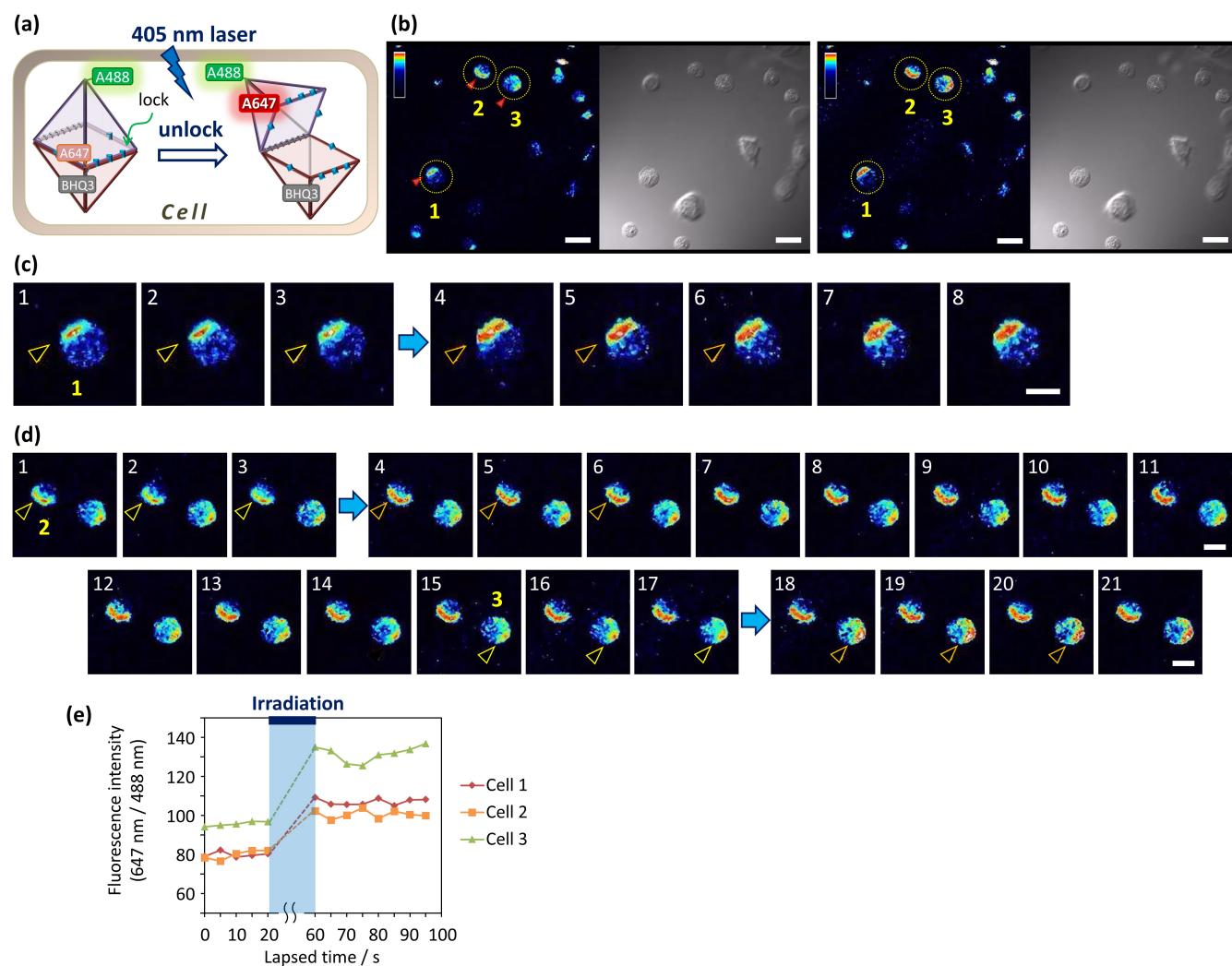


Figure 3. Single particle unlocking the NC with photoirradiation to single cell. (a) Unlocking the caged-NC with 405 nm laser irradiation. Caged-NC was labelled by Alexa 488 and the opening of the NC was monitor by recovery of fluorescence quenching of Alexa 647/BHQ-3 quencher during laser irradiation. Scale bar 20 μ m. (b) Fluorescence ratio images of cells for laser irradiation. Adjacent images are DIC images. Three individual cells (yellow circle 1-3) were irradiated sequentially. Before (left) and after individual laser irradiation to three cells (right). Fluorescence microscope images were reconstituted using ratio images of fluorescence intensity of Alexa 647/Alexa 488. Scale bar 10 μ m. (c) Time lapsed fluorescence ratio images of cell 1 before (image 3) and after (image 4) laser irradiation (40 s). Images were obtained at a rate of 0.2 frame/s (5 s interval for one image). Scale bar 10 μ m. (d) Sequential laser irradiation to individual cells. Time lapsed fluorescence ratio images of cells 2 and 3. Laser irradiation (40 s) to cell 2 and cell 3 was performed after image 3 and 17, respectively. Images were obtained at a rate of 0.2 frame/s (5 s interval for one image). Scale bar 10 μ m. (e) Change of fluorescence intensity of Alexa 647/Alexa 488 in cells 1-3 before and after laser irradiation.

thermore, the irradiation to the cell (cell 3; right side) independently and individually opened the caged-NC (image 18). Fluorescence intensity change in all three cells showed the similar tendency for the opening behavior (Figure 3e). The results indicate that the laser irradiation to the individual cells could open the caged-NC uptaken in the cell.

In conclusion, we have demonstrated that the photo-caged and locked nanocapsule has been constructed and can be delivered into the cell. Unlocking of the caged-NC was performed by photoirradiation and dynamic opening of the NC was measured and monitored by fluorescence quenching and recovery. The cells uptaken caged-NC were individually opened by single cell laser irradiation and monitored by imaging of fluorescence quenching and recovery. In the fluorescence images, the dynamic range of the quenching/recovery was not so significant (~5 times in Figure 1c). Therefore, the relative positioning of the dye and quencher in the NC is still needed to be improved. Using this NC system, gold nanoparticle can be included inside the large cavity as a cargo, which did not change the photoresponsive properties.¹⁹ Therefore, the caged-NC system could be applied for an intelligent delivery system for relatively large nanomaterials and proteins to the cells similar to the native virus system.

ASSOCIATED CONTENT

Supporting Information

Experimental procedure and fluorescence microscope image movie. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ACKNOWLEDGMENT

This work was supported by JSPS KAKENHI Fund for the Promotion of Joint International Research (Fostering Joint International Research (B) (Grant Numbers 18KK0139 and 16H06356). Financial supports from The Uehara Memorial Foundation, The Nakatani Foundation, and The Kyoto University Foundation to ME were also acknowledged.

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