Video Article

Spatiotemporally Controlled Nuclear Translocation of Guests in Living Cells Using Caged Molecular Glues As Photoactivatable Tags

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Abstract

The cell nucleus is one of the most important organelles as a subcellular drug-delivery target, since modulation of gene replication and expression is effective for treating various diseases. Here, we demonstrate light-triggered nuclear translocation of guests using caged molecular glue (^{Caged}Glue-R) tags, whose multiple guanidinium ion (Gu^+) pendants are protected by an anionic photocleavable group (butyrate-substituted nitroveratryloxycarbonyl; ^{BA}NVOC). Guests tagged with ^{Caged}Glue-R are taken up into living cells *via* endocytosis and remain in endosomes. However, upon photoirradiation, ^{Caged}Glue-R is converted into uncaged molecular glue (^{Uncaged}Glue-R) carrying multiple Gu^+ pendants, which facilitates the endosomal escape and subsequent nuclear translocation of the guests. This method is promising for site-selective nuclear-targeting drug delivery, since the tagged guests can migrate into the cytoplasm followed by the cell nucleus only when photoirradiated. ^{Caged}Glue-R tags can deliver macromolecular guests such as quantum dots (QDs) as well as small-molecule guests. ^{Caged}Glue-R tags can be uncaged with not only UV light but also two-photon near-infrared (NIR) light, which can deeply penetrate into tissue.

Video Link

The video component of this article can be found at https://www.jove.com/video/58631/

Introduction

The cell nucleus, which carries genetic information, is one of the most important organelles as a subcellular drug-delivery target, since modulation of gene replication and expression is effective for treating various diseases including cancer and genetic disorders[1,2,3]. For nuclear delivery of drugs, conjugation of peptide tags such as nuclear localization signals (NLS)[4,5,6] has been widely investigated. However, in order to reduce undesired side effects, spatiotemporal control of the nuclear translocation is necessary.

Previously, light-triggered translocation of proteins into the cell nucleus has been achieved using caged NLS[7,8,9]. NLS migrates into the cell nucleus by binding to cytoplasmic transport proteins[6]. In the reported methods, guest proteins bearing caged NLS are directly incorporated into the cytoplasm by microinjection[8] or expressed in the target cells using a genetic code expansion technique[9]. Therefore, a method that can achieve both cellular uptake and photo-induced nuclear translocation is advantageous for practical applications.

Herein, we describe light-triggered nuclear translocation of guests in living cells using dendritic caged molecular glue (^{Caged}Glue-R, **Figure 1**) tags. Water-soluble molecular glues[10,11,12,13,14,15,16,17,18,19,20,21,22,23] bearing multiple Gu^+ pendants have been previously developed, which tightly adhere to proteins[11,12,13,14,15,16,17], nucleic acids[18,19,20], phospholipid membranes[21], and clay nanosheets[22,23] through the formation of multiple salt bridges between their Gu^+ pendants and oxyanionic groups on the targets. The Gu^+ pendants of ^{Caged}Glue-R are protected by an anionic photocleavable group, butyrate-substituted nitroveratryloxycarbonyl (^{BA}NVOC). Guests tagged with ^{Caged}Glue-R are taken up into living cells *via* endocytosis and stay in endosomes (**Figure 2**). Upon photoirradiation, the ^{BA}NVOC groups of ^{Caged}Glue-R are detached to yield an uncaged molecular glue (^{Uncaged}Glue-R) carrying multiple Gu^+ pendants, which then facilitates the migration of the tagged guest into the cytoplasm followed by the cell nucleus (**Figure 2**). The ^{Caged}Glue-R tag can be uncaged by exposure to UV or two-photon near-infrared (NIR) light without serious phototoxicity. We demonstrate the spatiotemporally controlled nuclear delivery of macromolecular guests as well as small-molecule guests with ^{Caged}Glue-R tags, using quantum dots (QDs) and a fluorescent dye (nitrobenzooxadiazole; NBD), respectively, as examples.

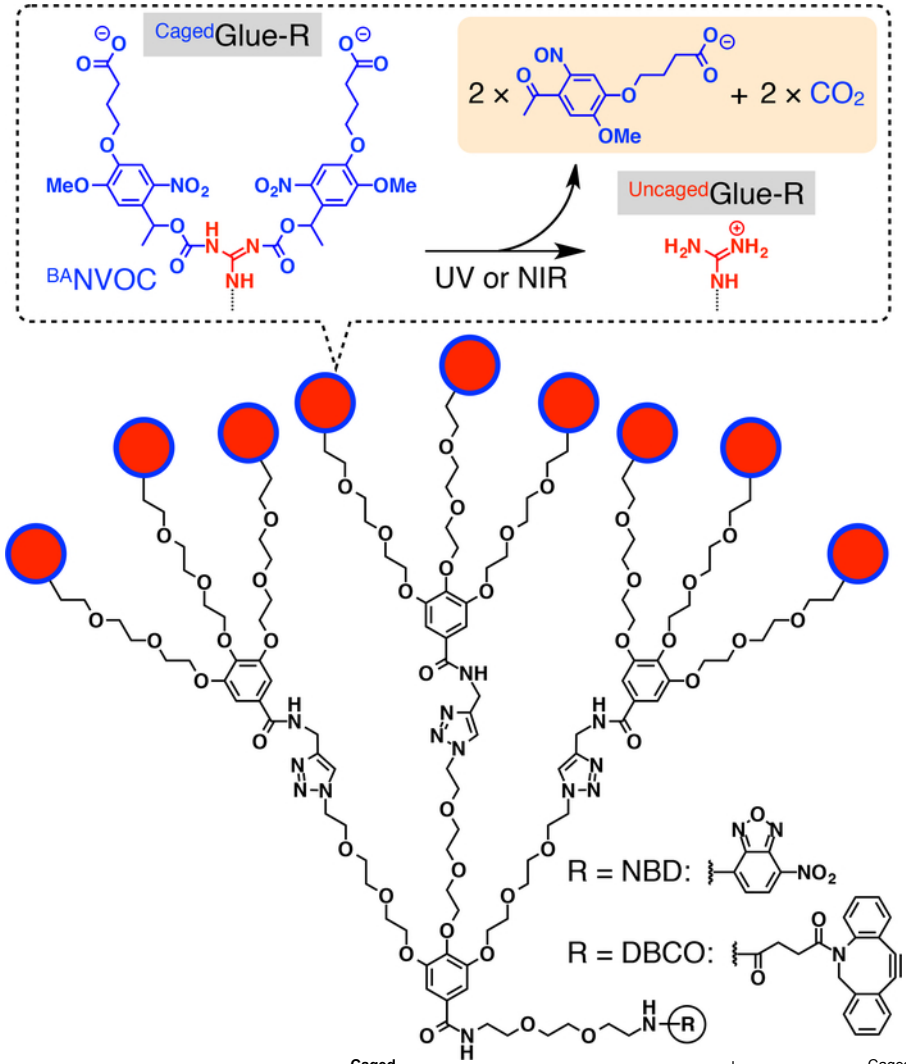


Figure 1: Schematic structures of ^{Caged}Glue-R. The 9 guanidinium ion (Gu^{+}) pendants of ^{Caged}Glue-R are protected by a butyrate-substituted nitroveratryloxycarbonyl (^{BA}NVOC) group. The ^{BA}NVOC groups are cleaved by irradiation with UV or two-photon NIR light. The focal core of ^{Caged}Glue-R is functionalized with either nitrobenzoxadiazole (NBD) or dibenzocyclooctyne (DBCO). Reprinted with permission from reference[20]. Please click here to view a larger version of this figure.

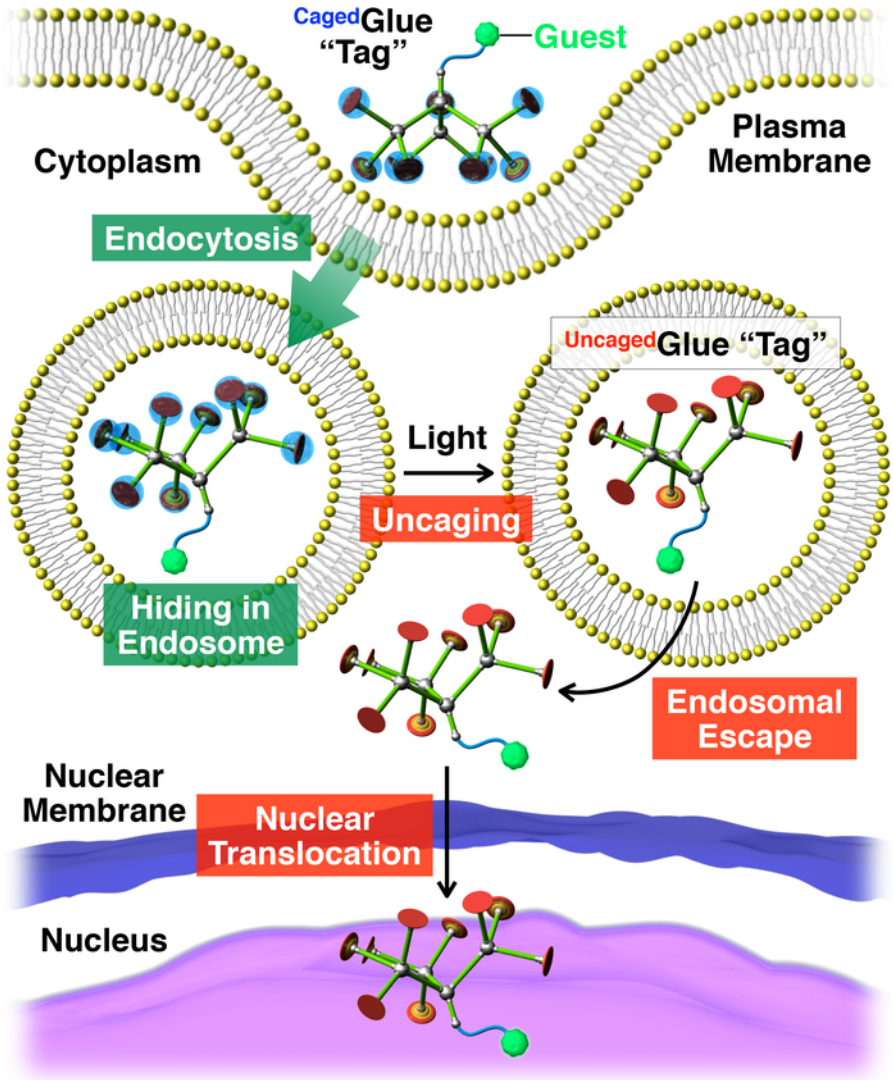


Figure 2: Schematic illustration of light-triggered nuclear translocation of guests conjugated with a ^{Caged}Glue-R tag. The guest/^{Caged}Glue-R conjugate is taken up into living cells *via* endocytosis. Upon photoirradiation, the ^{Caged}Glue-R tag is uncaged to yield an ^{Uncaged}Glue-R tag, which can facilitate endosomal escape of the tagged guest. Subsequently, the tagged guest migrates into the cell nucleus. Reprinted with permission from reference[20]. Please click here to view a larger version of this figure.

Protocol

1. Preparation of Guests with ^{Caged}Glue-R Tags

1. Prepare ^{Caged}Glue-NBD solution.
    1. Synthesize ^{Caged}Glue-NBD (**Figure 1**) following the procedures previously described[20].
    2. Prepare a stock solution of ^{Caged}Glue-NBD (10 mM) in dry dimethyl sulfoxide (DMSO).
    Note: Store the stock solution in dark. The solution can be diluted with aqueous buffers or cell culture media upon usage.

2. Prepare ^{Caged}Glue-QD solution.
    1. Synthesize ^{Caged}Glue-dibenzocylooctyne (^{Caged}Glue-DBCO) (**Figure 1**) following the procedures previously described[20].
    2. Prepare a stock solution of ^{Caged}Glue-DBCO (10 mM) in dry DMSO.
    3. For the preparation of ^{Caged}Glue-QD, first prepare azide-functionalized QDs (Azide-QD; **Figure 3**). Add 100 µL of a dimethyl formamide (DMF, 125 µM) solution of azide-PEG4-NHS ester (**Figure 3**) to 400 µL of a DMF (500 nM) solution of quantum dots (QDs) coated with amine-functionalized PEG (Amine-QD; **Figure 3**). Stir the mixture for 1 h at room temperature.
    4. Dialyze the resulting solution for 24 h against 500 mL of DMF using a regenerated cellulose membrane with 3,500 molecular weight cut-off (MWCO).
    5. Dilute the stock solution of ^{Caged}Glue-DBCO to 50 µM with DMF. Add 200 µL of the solution to the post-dialysis solution (**Figure 3**) and stir the mixture for 3 h at room temperature.
    6. Dialyze the resulting solution for 24 h against 500 mL of DMF using a regenerated cellulose membrane (25,000 MWCO).

7. Dilute the resulting solution to 200 nM with DMF.

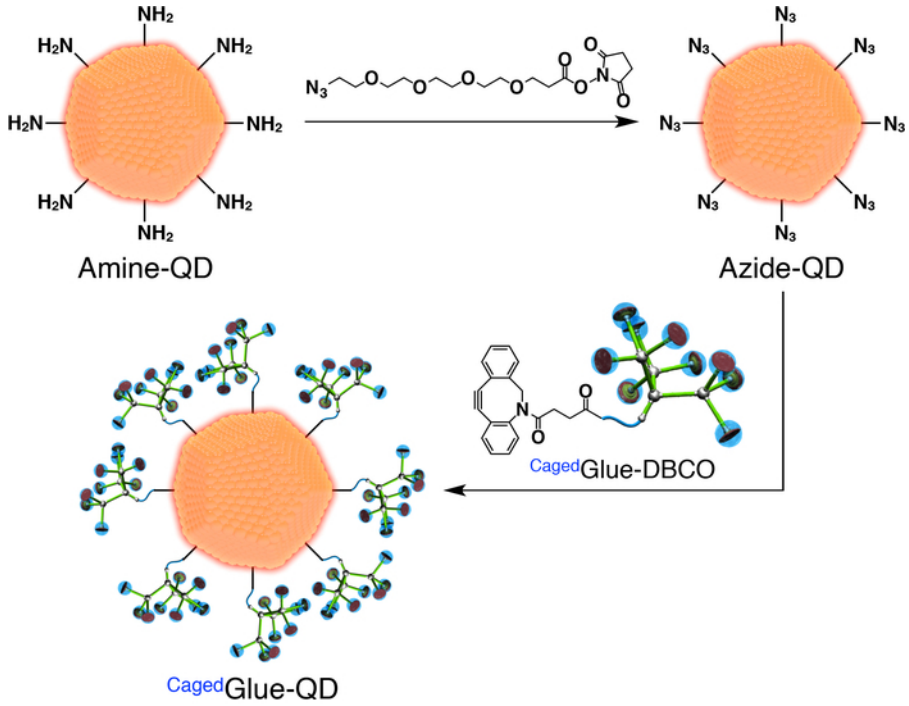


Figure 3: Schematic illustration of the preparation of ^{Caged}Glue-QD. Please click here to view a larger version of this figure.

2. Preparation of Hep3B Cell Sample for Microscopic Observations

1. Maintain human hepatocellular carcinoma Hep3B cells in Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum (FBS) at 37 °C under 5% CO_2.
2. Seed the cells the day before the experiment. Seed 5.0 × 10^3 Hep3B cells per well of an 8-chambered glass substrate in EMEM (10% FBS, 200 µL), and incubate the cell sample at 37 °C under 5% CO_2 for 24 h.
3. Remove the culture medium and rinse the cell sample with 100 µL of Dulbecco's phosphate buffer saline (D-PBS) twice.

3. Observation of Nuclear Translocation of Small-molecule Guests Triggered by UV Light

1. Supply the cell sample (prepared in step 2.3) with 200 µL of FBS-free EMEM containing ^{Caged}Glue-NBD (10 µM) and incubate the resulting cell sample at 37 °C under 5% CO_2 for 3 h.
   Note: Incubation of the cell sample in FBS-free EMEM for longer than 4 h causes serious cell damage.
2. Remove the culture medium and rinse the cell sample with 100 µL of D-PBS twice.
3. For visualization of the endosomes, supply the cell sample with 200 µL of EMEM (10% FBS) containing a red-fluorescent dye (*e.g.*, LysoTracker Red, 100 nM), and incubate the resulting cell sample at 37 °C under 5% CO_2 for 20 min. Remove the culture medium, and rinse the cell sample with 100 µL of D-PBS twice. Supply the cell sample with 200 µL of EMEM (10% FBS).
4. For nuclear translocation of ^{Caged}Glue-NBD, expose the cell sample to UV light for 2 min *via* an optical fiber using a 100-W xenon light source equipped with a 365 nm bandpass filter. For a reference cell sample without UV exposure, keep the cell sample in dark.
   Note: The lid of the glass substrates can be taken off for efficient UV exposure. Long-time exposure to UV light may cause cytotoxicity to the cells.
5. For visualization of the nuclei, add 1 µL of Hoechst 33342 (1 mg/mL) to the culture medium, and incubate the resulting cell sample at 37 °C under 5% CO_2 for 10 min.
6. Subject the cell sample to confocal laser scanning microscopy and record the micrographs upon excitation at 488 nm (λ_{obs} = 500-530 nm), 543 nm (λ_{obs} = 565-620 nm), and 710 nm (two-photon; λ_{obs} = 390-465 nm) for NBD, red-fluorescent dye, and Hoechst 33342, respectively.

4. Observation of Nuclear Translocation of Small-molecule Guests Triggered by Two-photon NIR Light

1. Supply the cell sample (prepared in step 2.3) with 200 µL of FBS-free EMEM containing ^{Caged}Glue-NBD (10 µM) and incubate the resulting cell sample at 37 °C under 5% CO_2 for 3 h.
2. Remove the culture medium and rinse the cell sample with 100 µL of D-PBS twice. Supply the cell sample with 200 µL of EMEM (10% FBS).
3. Subject the cell sample to confocal laser scanning microscopy and record the micrographs upon excitation at 488 nm (λ_{obs} = 500-530 nm).

4. For nuclear translocation of ^{Caged}Glue-NBD, irradiate the region including the cell of interest with a two-photon excitation laser (710 nm), installed as a light source in the microscope, for 2 min (30 s × 4). Observe the translocation as described in step 4.3.

5. Observation of Nuclear Translocation of Macromolecular Guests Triggered by UV Light

1. Supply the cell sample (prepared in step 2.3) with 200 µL of FBS-free EMEM containing ^{Caged}Glue-QD (10 nM), and incubate the resulting cell sample at 37 °C under 5% CO_2 for 3 h.
2. Remove the culture medium and rinse the cell sample with 100 µL of D-PBS twice. Supply the cell sample with 200 µL of EMEM (10% FBS).
3. For nuclear translocation of ^{Caged}Glue-QD, expose the cell sample to UV light for 2 min *via* an optical fiber using a 100-W xenon light source equipped with a 365 nm bandpass filter. For a reference cell sample without UV exposure, keep the cell sample in dark.
4. For visualization of the nuclei, add 1 µL of Hoechst 33342 (1 mg/mL) to the culture medium, and incubate the resulting cell sample at 37 °C under 5% CO_2 for 10 min.
5. Subject the cell sample to confocal laser scanning microscopy and record the micrographs upon excitation at 405 nm (λ_{obs} = 430-520 nm) and 488 nm (λ_{obs} = 625-680 nm) for Hoechst 33342 and QDs, respectively.

6. Cell Viability Assay

1. Maintain human hepatocellular carcinoma Hep3B cells in EMEM (10% FBS) at 37 °C under 5% CO_2.
2. Seed the cells the day before the experiment. Seed 5.0 × 10^3 Hep3B cells per well of a 96-well culture plate in EMEM (10% FBS, 200 µL), and incubate the cell sample at 37 °C under 5% CO_2 for 24 h.
3. Remove the culture medium and rinse the cell sample with 100 µL of D-PBS twice.
4. Supply the cell sample with 200 µL of FBS-free EMEM containing ^{Caged}Glue-NBD (0.1-100 µM) and incubate the resulting cell sample at 37 °C under 5% CO_2 for 3 h.
5. Expose the cell sample to UV light for 2 min *via* an optical fiber using a 100-W xenon light source equipped with a 365 nm bandpass filter. For an analogous cell sample without UV exposure, keep the cell sample in dark.
6. Add 10 µL of Cell Counting Kit-8 reagent (10 µL) to the culture medium and incubate the resulting cell sample at 37 °C under 5% CO_2 for 2 h.
7. Subject the cell sample to absorption spectroscopy (λ = 450 nm) using a microplate reader.

Representative Results

Before photoirradiation, Hep3B cells incubated with ^{Caged}Glue-NBD exhibited punctate fluorescence emission from their interior (λ_{ext} = 488 nm; **Figures 4A** and **4C**, green). An analogous micrograph was obtained upon excitation at 543 nm for red-fluorescent dye (**Figures 4B** and **4C**, red), indicating that ^{Caged}Glue-NBD localized in the endosomes. Accordingly, the fluorescence emission assignable to ^{Caged}Glue-NBD (**Figure 4D**, green) was observed outside the cell nucleus, which was visualized with Hoechst 33342 (λ_{ext} = 710 nm, two-photon; **Figure 4D**, blue). After UV irradiation, the cells emitted fluorescence due to NBD (**Figure 4E**, green) also from the nucleus (**Figure 4E**, blue), suggesting that ^{Caged}Glue-NBD was uncaged to yield ^{Uncaged}Glue-NBD, which migrated into the cytoplasm followed by the cell nucleus. Such nuclear translocation of ^{Caged}Glue-NBD can be induced site-selectively by two-photon NIR light (**Figure 5A** and **Movie 1**, white dashed circle). As shown in **Figure 5B** and **Movie 1**, ^{Caged}Glue-NBD in nonirradiated areas did not escape from the endosomes and remained as punctate fluorescence. No appreciable cytotoxicity was observed for the cells treated with ^{Caged}Glue-NBD before and even after the UV exposure (**Figure 6**).

The ^{Caged}Glue-R tags can also deliver macromolecular guests such as QDs into the cell nucleus. The QD/^{Caged}Glue-R conjugate (^{Caged}Glue-QD, **Figure 3**) can be taken up into Hep3B cells (**Figure 7A**). Visualization of the cell nucleus with Hoechst 33342 indicates that ^{Caged}Glue-QD remains outside of the nucleus before photoirradiation (**Figure 7A**). After UV exposure for 2 min, the fluorescence emission of QDs emerged in the nucleus (**Figures 7B** and **7C**). A cross-sectional image of the cells demonstrated that the fluorescence emission assignable to QDs is indeed emitted from inside the nucleus (**Figure 7D**).

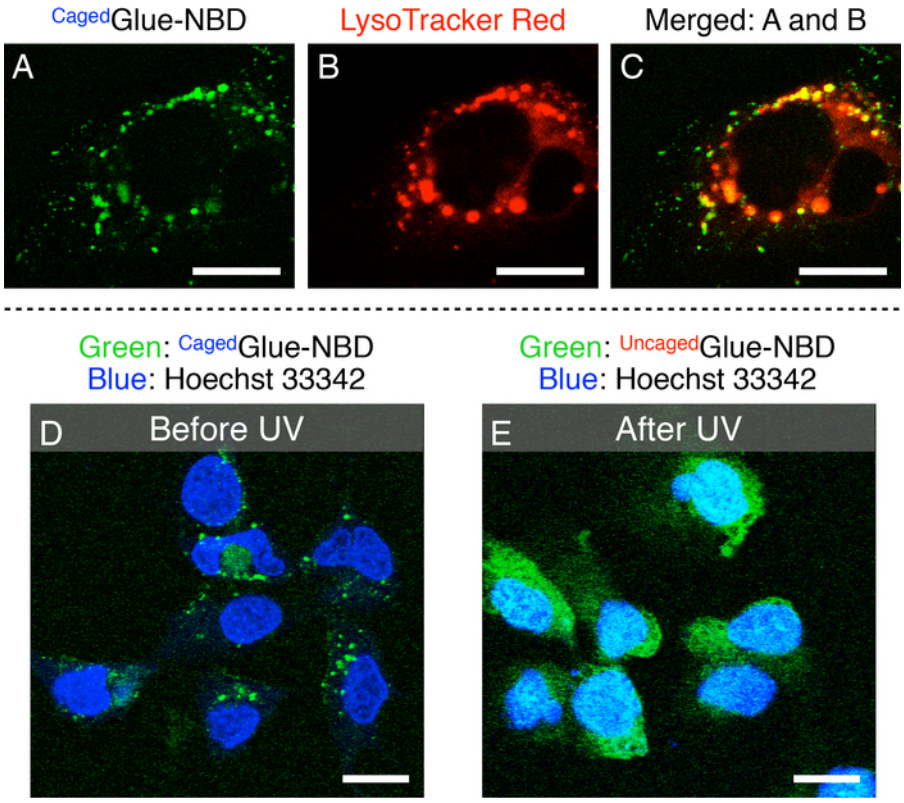


Figure 4: Endosomal escape and nuclear translocation of ^{Caged}Glue-NBD triggered by UV light. Confocal laser scanning micrographs of Hep3B cells after 3-h incubation at 37 °C in EMEM containing ^{Caged}Glue-NBD (10 μM) followed by rinsing with D-PBS. **(A, B)** Micrographs were recorded upon excitation at **(A)** 488 nm (λ_{obs} = 500-530 nm, green) and **(B)** 543 nm (λ_{obs} = 565-620 nm, red) after 20 min incubation in EMEM (10% FBS) containing red-fluorescent dye (100 nM). **(C)** Merged image of **(A)** and **(B)**. **(D, E)** Micrographs recorded upon excitation at 488 nm (λ_{obs} = 500-530 nm, green) and 710 nm (two-photon; λ_{obs} = 390-465 nm, blue). The Hep3B cells, treated with ^{Caged}Glue-NBD, were incubated at 37 °C in EMEM (10% FBS) containing Hoechst 33342 (5 μg/mL) before **(D)** and after **(E)** 2-min UV exposure at 365 nm. Scale bars = 20 μm. Reprinted with permission from reference[20]. Please click here to view a larger version of this figure.

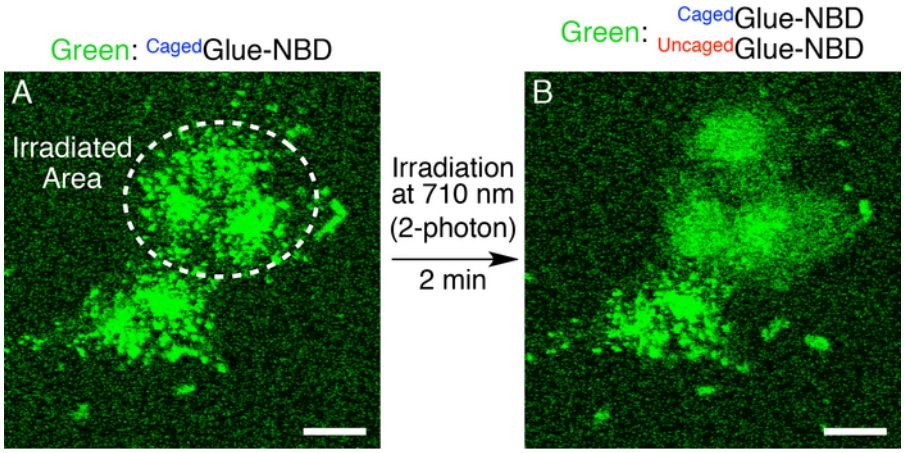


Figure 5: Nuclear translocation of ^{Caged}Glue-NBD triggered by two-photon NIR light. Confocal laser scanning micrographs of Hep3B cells after 3-h incubation at 37 °C in EMEM containing ^{Caged}Glue-NBD (10 μM) followed by rinsing with D-PBS. Micrographs were recorded upon excitation at 488 nm (λ_{obs} = 500-530 nm) before **(A)** and after **(B)** two-photon irradiation at 710 nm for 2 min (30 s × 4). The white dashed circle in **(A)** represents the irradiated area. Scale bars = 20 μm. Reprinted with permission from reference[20]. Please click here to view a larger version of this figure.

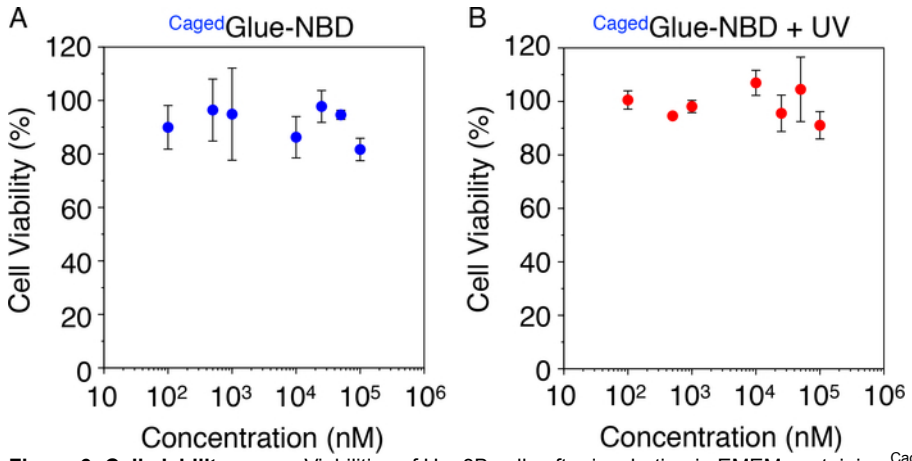


Figure 6: Cell viability assay. Viabilities of Hep3B cells after incubation in EMEM containing ^{Caged}Glue-NBD (0.1-100 μM) before **(A)** and after **(B)** 2-min UV exposure at 365 nm. Reprinted with permission from reference[20]. Please click here to view a larger version of this figure.

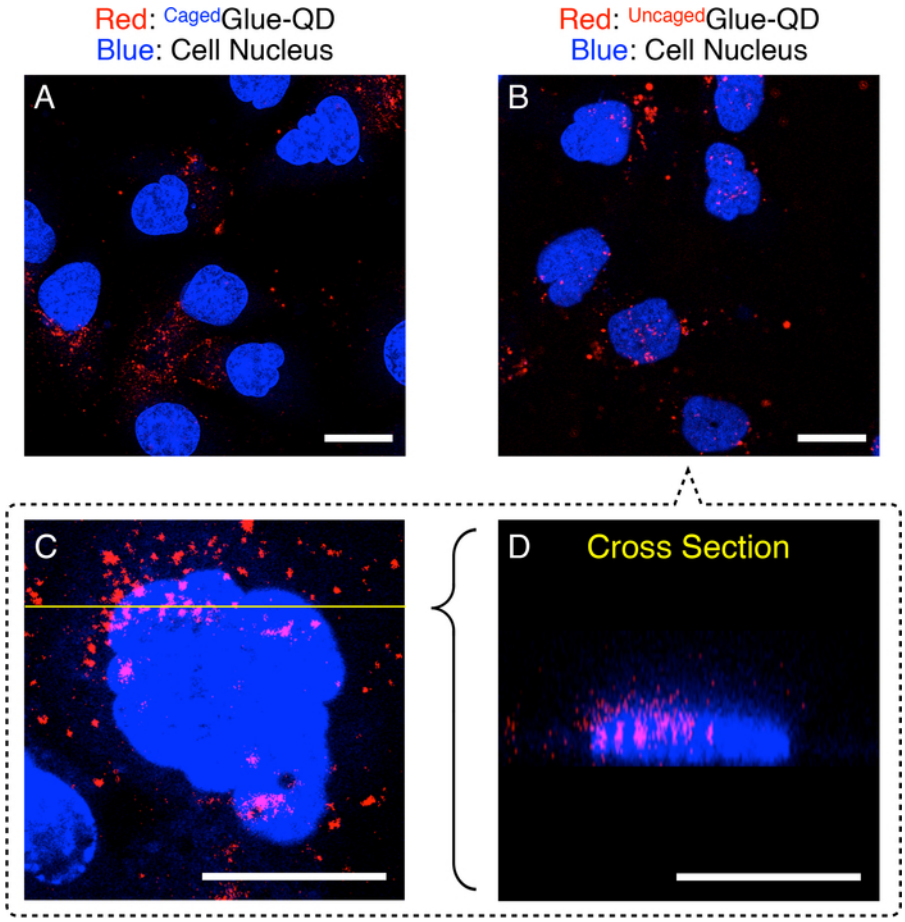
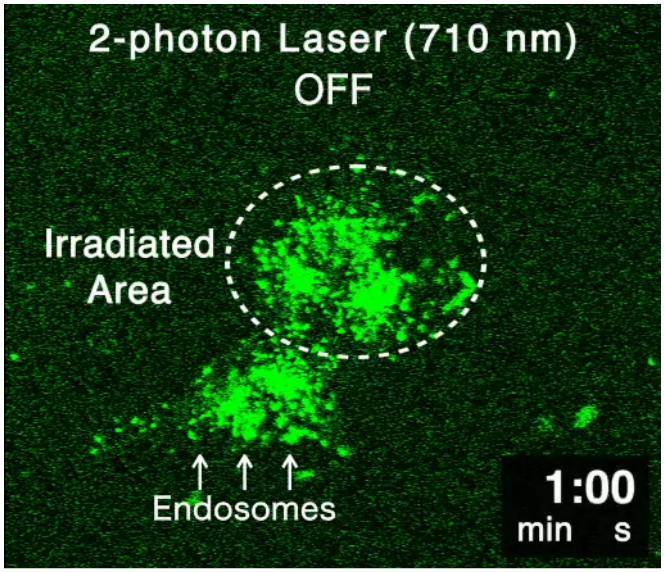


Figure 7: Nuclear translocation of ^{Caged}Glue-QD triggered by UV light. Confocal laser scanning micrographs of Hep3B cells upon excitation at 405 nm (λ_{obs} = 430-520 nm) and 488 nm (λ_{obs} = 625-680 nm). Hep3B cells were incubated at 37 °C for 3 h in EMEM containing ^{Caged}Glue-QD (10 nM), rinsed with D-PBS, and then incubated at 37 °C for 10 min in EMEM (10% FBS) containing Hoechst 33342 (5 μg/mL) before **(A)** and after **(B, C)** 2-min exposure to UV light at 365 nm. **(D)** Cross-sectional image along the yellow line in **(C)**. Scale bars = 20 μm. Reprinted with permission from reference[20]. Please click here to view a larger version of this figure.



Movie 1. Nuclear translocation of ^{Caged}Glue-NBD triggered by two-photon NIR light. Time-lapse confocal laser scanning microscopy of Hep3B cells after 3-h incubation at 37 °C in EMEM containing ^{Caged}Glue-NBD (10 μM) followed by rinsing with D-PBS. Micrographs were recorded with 3-s intervals upon excitation at 488 nm (λ_{obs} = 500-530 nm). The cells located in the white dashed circle were irradiated with two-photon NIR light at 710 nm for 2 min (30 s × 4). Reprinted with permission from reference[20]. Please click here to view this video. (Right-click to download.)

Discussion

Previous investigations of light-triggered translocation of proteins into the cell nucleus have been achieved using caged NLS[7,8,9]. As mentioned earlier, these methods require additional techniques to incorporate the NLS-tagged proteins into the cytoplasm. In contrast, our ^{Caged}Glue-R tag enables not only photo-induced nuclear translocation but also cellular uptake of the guests. This feature of the ^{Caged}Glue-R tag is advantageous for *in vivo* applications.

The ^{Caged}Glue-R tag can deliver macromolecular guests such as QDs into the cell nucleus. The QDs employed here are larger in diameter (D_H = 15-20 nm) than the nuclear pores (~5 nm)[24], suggesting the possibility to deliver other macromolecules that cannot passively diffuse into the nucleus. Protocols for functionalization of biomacromolecular surfaces with azide groups are well established[25]; in addition, synthesis of ^{Caged}Glue-R tags bearing other functional groups such as maleimides, *N*-hydroxysuccinimide (NHS) esters, and so forth as an anchoring unit will broaden the applicability of ^{Caged}Glue-R tags to various biomacromolecules.

Needless to say, the critical step of this method is the incorporation of the guests tagged with ^{Caged}Glue-R *via* endocytosis. The efficiency of the cellular uptake of the tagged guests depends on their concentration and the incubation time. If guests of interest, when tagged with ^{Caged}Glue-R, are endocytosed inefficiently, incubate the cells longer with higher concentration of the tagged guests. A possible alternative is to increase the number of ^{Caged}Glue-R incorporated into the guest.

The guests used in this protocol are covalently conjugated to the ^{Caged}Glue-R tag. This is a potential disadvantage especially for delivery of small-molecule ligands, since their binding to the target biomolecules may be inhibited by the bulky dendritic tag. Incorporation of a stimuli-responsive linker[26] between the ^{Caged}Glue-R tag and the guest molecule may allow release of the guests after nuclear translocation.

In summary, we demonstrated light-triggered nuclear translocation of guests using photoactivatable caged molecular glue (^{Caged}Glue-R) tags. The guests tagged with ^{Caged}Glue-R are taken up into living cells *via* endocytosis and remain in the endosomes. Upon photoirradiation, ^{Caged}Glue-R is converted to ^{Uncaged}Glue-R, which facilitates the endosomal escape and subsequent nuclear translocation of the guests. Further long-time observations may be important to investigate the fate of the guests that remain in the endosomes as well as those delivered into the cell nucleus. Spatiotemporally controlled gene expression using ^{Caged}Glue-R tags is an interesting subject worthy of further investigation.

Disclosures

The authors have nothing to disclose.

Acknowledgements

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