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Photoreconfigurable Supramolecular Nanotube

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Supporting Information

ABSTRACT: A photoreconfigurable bionanotube was developed by Mg^{2+}-induced supramolecular polymerization using $GroEL_{SP}$, a mutant barrel-shaped chaperonin protein bearing multiple photochromic spiropyran (SP) units at its apical domains. Upon exposure to UV light, the nonionic SP units isomerize into ionic merocyanine (MC) to afford $GroEL_{MC}$, which is capable of polymerizing with $MgCl_2$. The resultant nanotube (NT) is stable as a result of multiple MC···Mg^{2+}···MC bridges but readily breaks up into short NTs, including monomeric $GroEL_{SP}$, by the reverse (MC → SP) isomerization mediated by visible light. When this scission mixture is exposed to UV light, long NTs are reconfigured. A Förster resonance energy transfer (FRET) study revealed that NTs in the dark maintain their sequential integrity. However, when exposed to visible and UV light successively, the NTs lose their sequential memory as a result of intertubular reshuffling of the constituent $GroEL_{MC}$ units.

In biological systems, stimuli-responsive supramolecular polymerization and depolymerization play a crucial role in triggering biological events. Examples include a tubulin dimer that polymerizes non-covalently when bound to guanosine 5′-triphosphate (GTP) to afford microtubules, which in turn undergo depolymerization to regenerate the tubulin dimer upon hydrolysis of bound GTP into guanosine 5′-diphosphate (GDP).[1] This reconfigurational process gives rise to intracellular transport and cell division. We herein report a novel bionanotube (NT) with a photoreconfigurable nature (Figure 1b). Although this NT assembly is highly stable against dissociation, it readily breaks up into short oligomers upon exposure to visible light. However, when the resultant scission mixture is exposed to UV light, long NTs are retrieved. Photoreconfigurable bionanotubes are attractive because of their potential to deliver guests in desired time and space domains in a noncontact manner. However, no photoreconfigurable bionanotubes[2] have been reported to date. Even in a larger library of synthetic supramolecular nanotubes,[3] successful examples are highly limited.[4]

The monomer employed for the present study was $GroEL_{MC}$ (MW 800 kDa), a mutant of the molecular chaperone GroEL[5,6] carrying at its apical domains photochromic merocyanine (MC) units (Figure 1a). In 2009, we reported that this molecularly engineered chaperonin protein undergoes supramolecular polymerization to give long NTs in the presence of Mg^{2+} via the formation of multiple MC···Mg^{2+}···MC bridges.[7a] Since MC

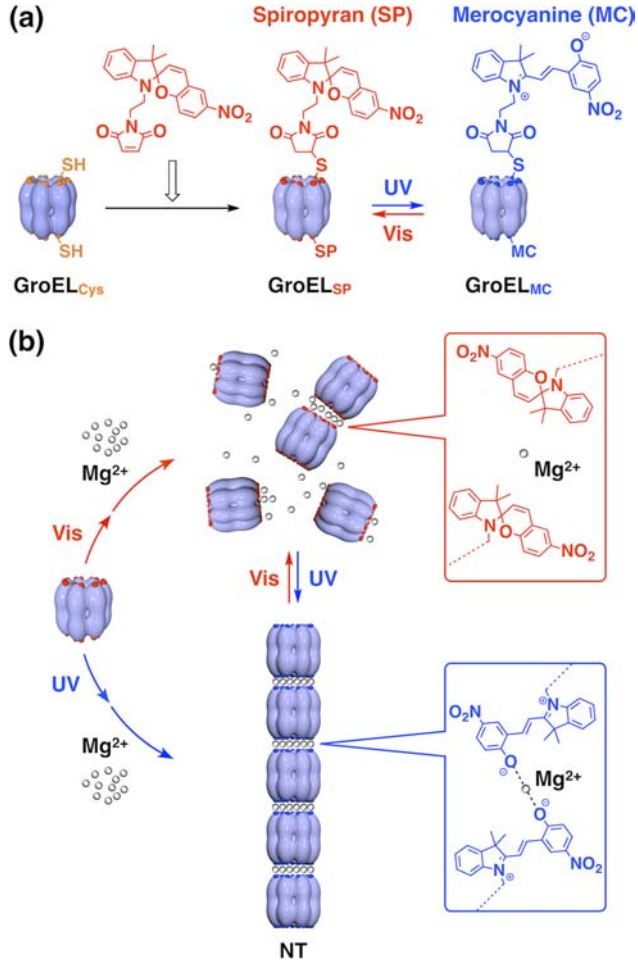

Figure 1. Schematic illustrations of (a) the preparation of $GroEL_{SP}$ carrying multiple spiropyran (SP) units at its apical domains and the reversible photochemical transformation of $GroEL_{SP}$ into $GroEL_{MC}$ carrying merocyanine (MC) units and (b) light-mediated formation/dissociation of its nanotubular assembly (NT).

is reversibly convertible into nonionic spiropyran (SP) photochemically,[8] we envisioned that irradiation with UV and visible light may give rise to the formation and dissociation of NTs, respectively, in a noncontact manner (Figure 1b). However, despite the occurrence of the MC → SP isomerization under

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visible light, NTs once formed did not break up into short-chain oligomers. As a typical example, NTs were prepared at 25 °C upon 15 min UV exposure (λ = 280 ± 10 nm) of a Tris-HCl buffer (25 mM) solution of $GroEL_{SP}$ (2 μM) containing $MgCl_2$ (5 mM). The resultant mixture containing long NTs (blue curve in Figure S1a in the Supporting Information) was then exposed to visible light (λ > 400 nm) for 15 min at 25 °C.[9] Although the absorption spectral features before and after the exposure to visible light clearly indicated the isomerization of MC into SP (Figure S1b) at the apical domains of GroEL, no substantial change in the size-exclusion chromatography (SEC) results was observed (red curve in Figure S1a).[9] As reported previously, in Tris-HCl buffer containing $MgCl_2$, $GroEL_{SP}$ polymerizes even in the dark as a result of a spontaneous transformation of $GroEL_{SP}$ into $GroEL_{MC}$.[10] Furthermore, when the connecting Mg^{2+} ions (5 mM) were removed by the action of EDTA (25 mM), the NTs obtained in the dark were cut into short NTs including monomeric $GroEL_{MC}$ (Figure S2a).[7a] However, as observed by SEC (Figure S2b),[9] NTs prepared under irradiation with UV light were hardly cut into short-chain oligomers upon treatment with EDTA. After struggling, we eventually noticed a literature report that an MC analogue generates radical species upon photoexcitation.[11] If radicals are really generated in our system, the $GroEL_{MC}$ units might be covalently cross-linked,[12] preventing dissociation of the resultant NTs. Having this possibility in mind, we carried out the UV-mediated polymerization of $GroEL_{MC}$ in the presence of dithiothreitol (DTT), a representative radical scavenger for biological studies,[13] and confirmed that the NTs thus formed were broken up by the action of EDTA.

According to a procedure analogous to that reported previously,[7] $GroEL_{SP}$ was prepared by the reaction of $GroEL_{Cys}$ with a SP-appended maleimide derivative (Figure 1a). By means of Ellman's test, we confirmed nearly complete consumption of the apical cysteine SH groups of $GroEL_{Cys}$. As shown in Figure 2a, when a Tris-HCl buffer (25 mM) solution of $GroEL_{SP}$ (2 μM) containing a mixture of $MgCl_2$ (5 mM) and DTT (1 mM) was continuously exposed to visible light (λ > 400 nm) at 25 °C, the polymerization hardly proceeded, with only a small change in the SEC results (black to red curves). Accordingly, electronic absorption spectroscopy (black and red curves in Figure 2b) indicated that $GroEL_{SP}$ remained substantially unchanged despite the presence of a concomitant isomerization process to $GroEL_{MC}$ in the buffer (vide ante).[10] However, when this solution was exposed to UV light (λ = 280 ± 10 nm) for 15 min at 25 °C, electronic absorption spectroscopy clearly showed the transformation of $GroEL_{SP}$ into $GroEL_{MC}$ (blue curve in Figure 2b). Accordingly, the SEC trace of the irradiated mixture displayed an elution peak in a higher molecular weight region due to the formation of NTs (blue curve in Figure 2a). Consistent with this SEC profile, transmission electron microscopy (TEM) clearly visualized the presence of NTs as long cylindrical objects (Figure 2d). In sharp contrast, when the mixture was kept exposed to visible light, only monomeric GroEL and its short-chain oligomers were observed (Figure 2c; for a reference TEM image of $GroEL_{SP}$, see Figure 3f).

The NTs thus formed were photocleavable with visible light (Figure 1b). When a polymerization mixture at 25 °C in the presence of DTT was exposed to visible light (λ > 400 nm) for 15 min, its SEC trace showed only an oligomeric fraction containing the monomer up to hexamers (red solid curve in Figure 3a), whereas the fraction containing long NTs observed before the irradiation with visible light (blue solid curve in Figure 3a)

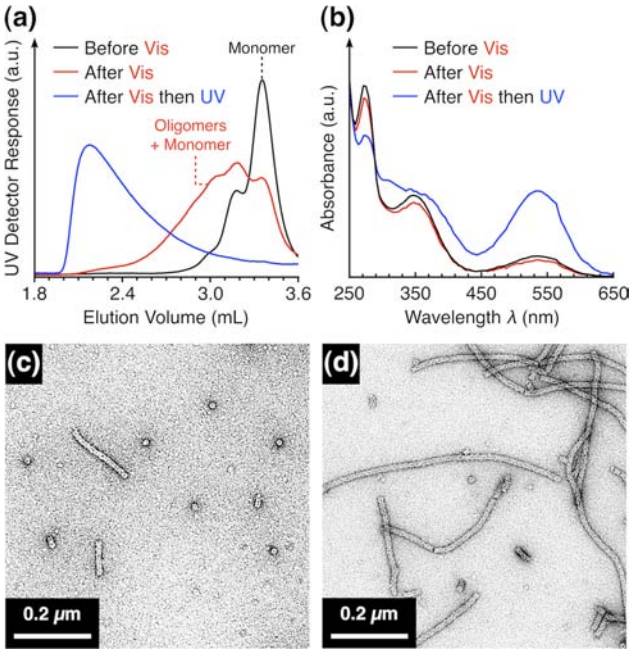

Figure 2. (a) SEC traces (monitored at λ = 280 nm, 25 °C) and (b) absorption spectra (photodiode array detector, 25 °C) of a Tris-HCl buffer (25 mM) solution of $GroEL_{SP}$ (2 μM) containing a mixture of $MgCl_2$ (5 mM) and DTT (1 mM) before (black curves) and after (red curves) a 15 min exposure to visible light and a subsequent 15 min exposure to UV light (blue curves) at 25 °C. (c, d) TEM images of air-dried samples of a Tris-HCl buffer (25 mM) solution of $GroEL_{SP}$ (2 μM) containing a mixture of $MgCl_2$ (5 mM) and DTT (1 mM) after (c) a 15 min exposure to visible light and (d) a subsequent 15 min exposure to UV light at 25 °C.

disappeared completely. Accordingly, an absorption spectral change (blue and red solid curves in Figure S3)[9] assignable to the transformation of $GroEL_{MC}$ into $GroEL_{SP}$ (Figure 1a) took place. Meanwhile, subsequent exposure of the scission mixture to UV light (λ = 280 ± 10 nm) in turn resulted in the recovery of the original SEC trace and absorption spectral profile (blue broken curves in Figures 3a and S3, respectively).[9] Consistently, TEM indicated that upon irradiation with visible light, the long NTs (Figure 3c) broke up into short-chain oligomers, including GroEL monomers (Figure 3d). Upon subsequent exposure to UV light, these short NTs were reconnected, eventually affording NTs longer than 1.5 μm (Figure 3e). Dynamic light scattering (DLS) analysis (Figure 3b) allowed us to confirm the photoreconfigurable nature of the chaperonin NTs (Figure 1b). Thus, 15 min exposure of a buffer solution of long NTs to visible light (λ > 400 nm) in the presence of DTT resulted in a complete shift of the DLS histogram (blue solid curve) to a smaller hydrodynamic volume region (red solid curve). Subsequent exposure of this scission mixture to UV light enabled nearly a perfect recovery of the original DLS histogram (blue broken curve). Such a light-mediated reversible change in hydrodynamic volume could be repeated multiple times.

The NTs must be intrinsically dynamic since they are formed by non-covalent interactions. However, on the basis of their SEC profiles, they likely possess substantial thermodynamic stability. By taking advantage of the guest-capturing function of GroEL, we confirmed that the NTs hardly reshuffle their constituent monomer units (Figure 4a). GroEL is known to capture denatured proteins in its 2.2 nm deep apical-domain craters.[5c] It is also noteworthy that guest proteins, if irreversibly denatured,

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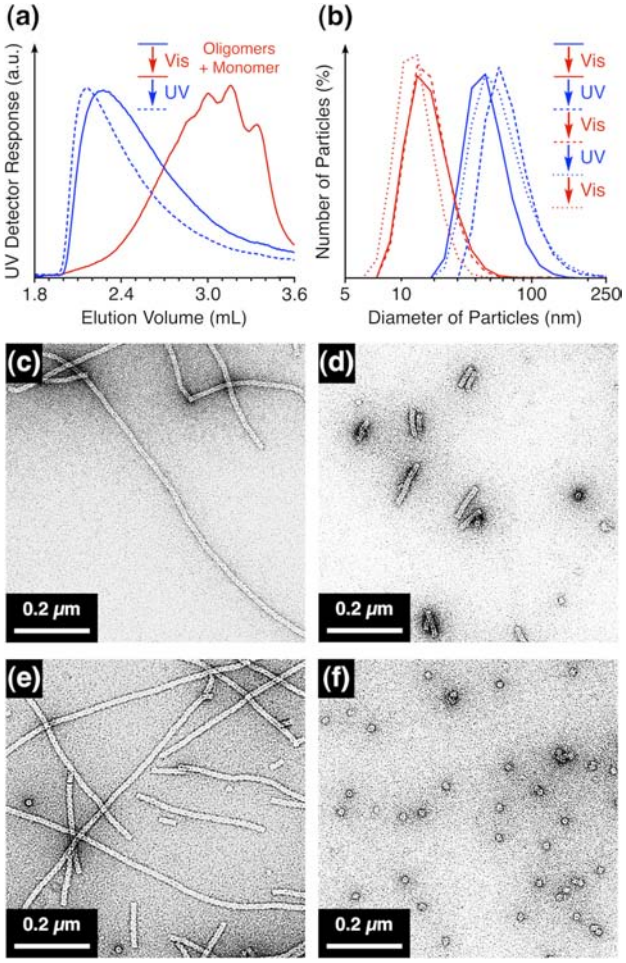

Figure 3. (a) SEC traces (monitored at λ = 280 nm, 25 °C) of a Tris-HCl buffer (25 mM) solution of $GroEL_{MC}$ (2 μM) containing a mixture of $MgCl_2$ (5 mM) and DTT (1 mM) after 15 min exposure to UV light (blue solid curve) followed by 15 min exposure to visible light (red solid curve) and subsequent 15 min exposure to UV light (blue broken curve) at 25 °C. (b) DLS profiles of NTs upon sequential exposure to visible → UV → visible → UV → visible light under conditions identical to those for (a). (c–e) TEM images of air-dried samples of (c) NTs prepared by 15 min exposure of a Tris-HCl buffer (25 mM) solution of a mixture of $GroEL_{MC}$ (2 μM) and $MgCl_2$ (5 mM) to UV light in the presence of DTT (1 mM), (d) oligomers formed by 15 min exposure of (c) to visible light, and (e) NTs formed by subsequent 15 min exposure of (d) to UV light at 25 °C. (f) Reference TEM image of an air-dried sample of $GroEL_{sp}$.

are not released from GroEL once captured.[14] On the basis of this, we prepared two fluorescently labeled guests from irreversibly denatured α-lactalbumin (LA_{denat}),[7b,9] $^{Rhd}LA_{denat}$ labeled with 5-carboxytetramethylrhodamine, a FRET donor (D), and $^{Alx}LA_{denat}$ labeled with Alexa Fluor 647, a FRET acceptor (A).[15] Each of these guest proteins was then allowed to hybridize with $GroEL_{MC}$,[9] affording $GroEL_{MC}\supset{}^{Rhd}LA_{denat}$ ($^{D}GroEL_{MC}$) and $GroEL_{MC}\supset{}^{Alx}LA_{denat}$ ($^{A}GroEL_{MC}$) (box in Figure 4a). Next, the resultant fluorescently labeled $GroEL_{MC}$ monomers were homopolymerized with $MgCl_2$ in the presence of DTT (1 mM), affording ^{D}NT and ^{A}NT. For comparison, we also prepared $^{DA}NT_{copoly}$, a statistical copolymer of $^{D}GroEL_{MC}$ and $^{A}GroEL_{MC}$, by adding $MgCl_2$ (5 mM) into an equimolar mixture of the monomers containing DTT (1 mM). As shown in Figure 4b, the fluorescence spectrum (λ_{ext} = 520 nm) of an equimolar mixture of ^{D}NT and ^{A}NT ([ii], black curve) differed

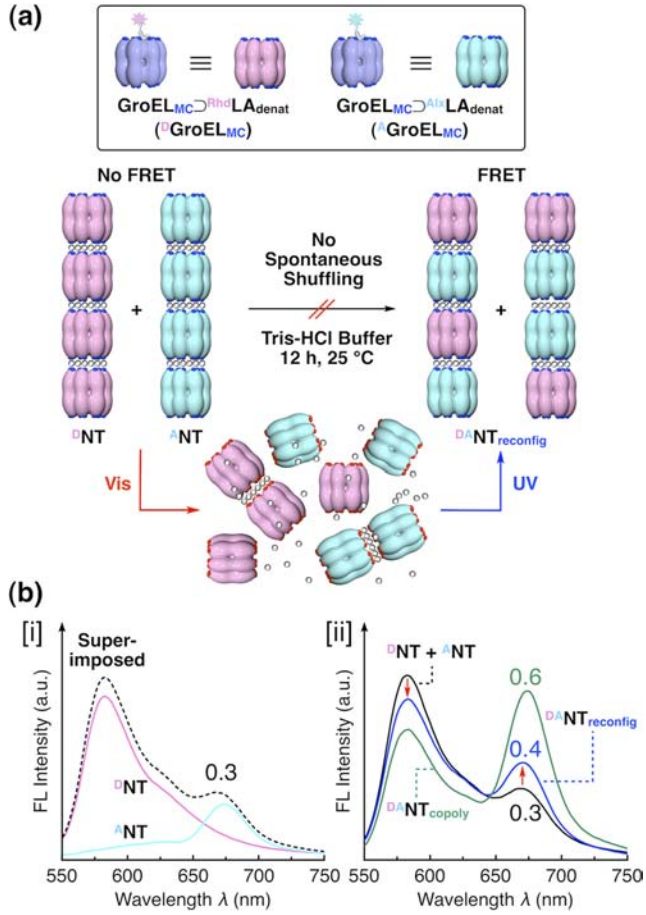

Figure 4. (a) Schematic illustrations of $^{D}GroEL_{MC}$ (pink) and $^{A}GroEL_{MC}$ (cyan) prepared by hybridization of $GroEL_{MC}$ with the irreversibly denatured α-lactalbumin guests $^{Rhd}LA_{denat}$ and $^{Alx}LA_{denat}$ labeled with 5-carboxytetramethylrhodamine (Rhd), a FRET donor (D) and Alexa Fluor 647 (Alx), a FRET-acceptor (A), respectively (box at top), and $^{DA}NT_{reconfig}$ (right) formed from an equimolar mixture of guest-containing ^{D}NT and ^{A}NT composed of $^{D}GroEL_{MC}$ and $^{A}GroEL_{MC}$, respectively (left), by photoreconfiguration (visible-light-induced scission followed by UV-light-induced reassembly). (b) Fluorescence spectra (λ_{ext} = 520 nm, 25 °C) of Tris-HCl buffer (25 mM) dispersions of [i] ^{D}NT (pink curve) and ^{A}NT (cyan curve) and their superimposed spectral image (black broken curve) and [ii] an equimolar mixture of ^{D}NT and ^{A}NT before (black curve) and after (blue curve) 15 min exposure to visible light followed by 15 min exposure to UV light at 25 °C and the statistical copolymer $^{DA}NT_{copoly}$ obtained from an equimolar mixture of $^{D}GroEL_{MC}$ and $^{A}GroEL_{MC}$ (green curve). Numbers in Figure 4b represent values of $I_A/(I_D + I_A)$, where I_D and I_A denote the fluorescence intensities at 581 and 671 nm upon photoexcitation at 520 nm. Conditions for the preparation of ^{D}NT, ^{A}NT, and $^{DA}NT_{copoly}$ were identical to those described for Figure 2.

from that of $^{DA}NT_{copoly}$ ([ii], green curve) but was essentially identical to a superimposed image ([i], black broken curve) of the fluorescence spectra of the homopolymers ^{D}NT ([i], pink curve) and ^{A}NT ([i], cyan curve). In fact, the $I_A/(I_D + I_A)$ value of 0.6 for $^{DA}NT_{copoly}$ (where I_D and I_A represent the fluorescence intensities at 581 and 671 nm, respectively) was obviously larger than those for the equimolar mixture of ^{D}NT and ^{A}NT (0.3) and the superimposed image of their fluorescence spectra (0.3). Therefore, FRET occurs efficiently from $^{D}GroEL_{MC}$ to $^{A}GroEL_{MC}$ in $^{DA}NT_{copoly}$, whereas no FRET takes place between ^{D}NT and ^{A}NT. The occurrence of FRET in $^{DA}NT_{copoly}$ is reasonable, since the immobilized Rhd and Alx groups in adjacent $^{D}GroEL_{MC}$

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and $^{A}GroEL_{MC}$ units (estimated distance ~4.4 nm) are located within the allowed distance of energy transfer via the Förster mechanism (6.9 nm).[15] Just in case, we incubated an equimolar mixture of ^{D}NT and ^{A}NT for 12 h in the buffer, and no change in the fluorescence spectrum resulted. This observation indicates the important notion that ^{D}NT and ^{A}NT hardly reshuffle their constituent $GroEL_{MC}$ units dynamically (Figure 4a). In other words, the NTs maintain their sequential integrity. Since the NTs are reconfigurable by light (vide ante, Figure 1b), the equimolar mixture of ^{D}NT and ^{A}NT was allowed to undergo depolymerization (black and red curves in Figure S4) upon exposure to visible light (>400 nm, 15 min) followed by repolymerization (blue curve in Figure S4) upon exposure to UV light (280 ± 10 nm, 15 min).[9] As expected, we observed a clear spectral change (black to blue) due to FRET (Figure 4b[ii]) with an enhancement of I_A at the expense of I_D [$I_A/(I_D + I_A) = 0.4$]. This value is reasonable considering that the photodissociated mixture, as estimated by the SEC trace (red curve in Figure S4), contained only 32% of the monomer/dimer fraction eligible for the recopolymerization. Therefore, the NTs upon photoreconfiguration ($^{DA}NT_{reconfig}$; Figure 4a) lost their original sequential memory.

In conclusion, we have demonstrated that nanotubes composed of chaperonin units that are non-covalently connected by a photochromic motif (Figure 1) are the first supramolecular NTs with a photoreconfigurable nature. As reported recently, these NTs, upon proper surface modification, can serve as a potent nanocarrier responsive to ATP.[7b] Since light is a noninvasive stimulus, the photoresponsive nature demonstrated in the present work provides the chaperonin NTs with an additional handle that may allow guest delivery in desired time and space domains.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Details of experimental procedures and spectral data, including the preparation of $GroEL_{SP}$ and $GroEL_{MC}$ and their hybridization with $^{Dye}LA_{denat}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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