Biomolecular robotics for chemomechanically driven guest delivery fuelled by intracellular ATP

Shuvendu Biswas, Kazushi Kinbara, Tatsuya Niwa, Hideki Taguchi, Noriyuki Ishii,

Sumiyo Watanabe, Kanjiro Miyata, Kazunori Kataoka and Takuzo Aida*

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1. Introduction

1.1. Surgical nanomachines

• Detection of biological signals (ex. hormone, cytokine, ATP) => Cure for wounded tissues

• Main problem of surgical nanomachines is how to supply a power source to them in the human body.

• ATP (adenosine triphosphate): biological fuel, Concentration of intracellular ATP is very high, in the range of 1–10 mM, much greater than that of extracellular ATP (< 5 μM). => Intracellular drug delivery

1.2. Chaperonin GroEL: A barrel-shaped protein[1]

• Prevention of aggregation and promotion of efficient folding of low-dimensional (1D or 2D) protein (Figure 1)

• GroEL can contain molecules inside the barrel.

• GroEL changes the structure by ATP-induced mechanical motion (open and close motion).

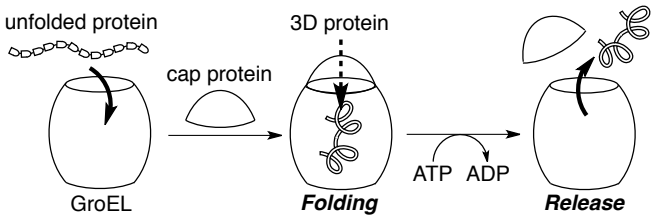

Figure 1. 3D folding of protein by GroEL.

1.3. Metal ion-induced 1D assembly of GroEL[2]

• Mutation of GroEL

Introduction of cysteine residues on the entrance of the barrel (Figure 2)

• Modification the cysteine residues with photochromic merocyanine (MC) (Figure 2)

• Formation of nanotube by supramolecular polymerization of the $GroEL_{MC}$ **via coordinate bonds between MC and divalent Mg^{2+} ions** ($[Mg^{2+}]$ / [MC] = 300) (Figure 3a, 3b, 3c)

• Other divalent cations such as Ca^{2+}, Mn^{2+}, Co^{2+} and Zn^{2+} triggered the assembly, but monovalent cations such as Na^{+}, K^{+} and Cs^{+} hardly induced the assembly.

•EDTA (a metal ion chelator) treatment

The long nanotubes were cut into short-chain oligomers or monomer (Figure 3d).

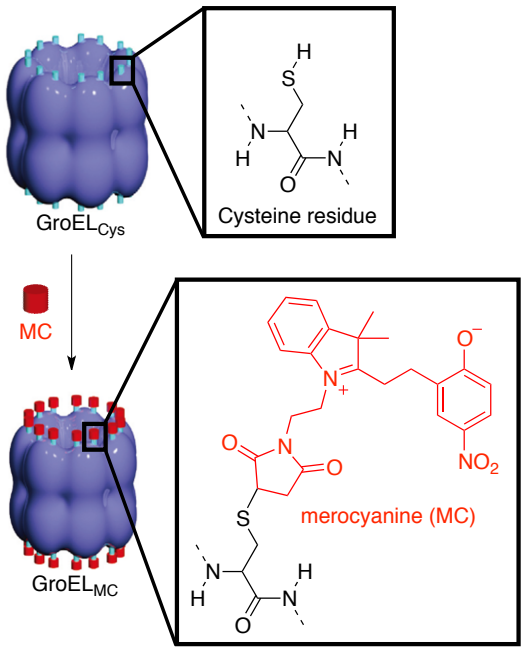

Figure 2. Modification of mutant $GroEL_{Cys}$ with merocyanine.

• Degree of functionalization of SH group of the cysteine residues with MC at the apical domains of $GroEL_{Cys}$ (SH/MC-conversion) significantly affects the polymerization profiles of the resultant $GroEL_{MC}$.

– SH/MC-conversion < 56% => no polymerization

– Long nanotubes (up to 2.5 μm, SH/MC-conversion = 100%) were observed when the SH/MC-conversion was high due to an enhancement of multivalency of the $GroEL_{MC}$ connection.

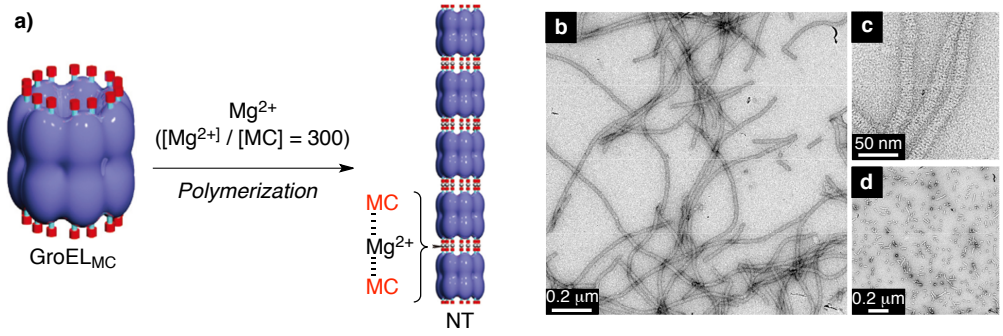

Figure 3. $GroEL_{MC}$ nanotube. (a) Schematic illustration of Mg^{2+}-induced 1D assembly of $GroEL_{MC}$. TEM micrographs of one-dimensionally assembled $GroEL_{MC}$ after mixing with 5 mM $MgCl_2$ (b,c) before and (d) after mixing with 25 mM EDTA.

2. Results and Discussion

2.1. ATP-responsive dissociation of $GroEL_{MC}$ nanotubes

• $GroEL_{MC}$ nanotubes readily break up through the action of ATP into short-chain oligomers (Figure 4a).

• The nanotubes have almost the same ATPase activity as $GroEL_{Cys}$ monomer (Figure 4b inset).

• **ATP induce a conformational change of GroEL.[1]**

=> MC–Mg^{2+}–MC bonds are cleaved.

=> Dissociation of the nanotubes

• Measured by size-exclusion chromatography (SEC), after 30 min incubation with ATP, three kinds of sharp signals that are corresponding to $GroEL_{MC}$ monomer, dimer and trimer were observed (Figure 4b).

• The nanotubes are not equilibrated with the monomer considering no disruption under repeated chromatographic treatment.

• Effect of ATP to the dissociation is 250 times higher than that of removing Mg^{2+} by EDTA.

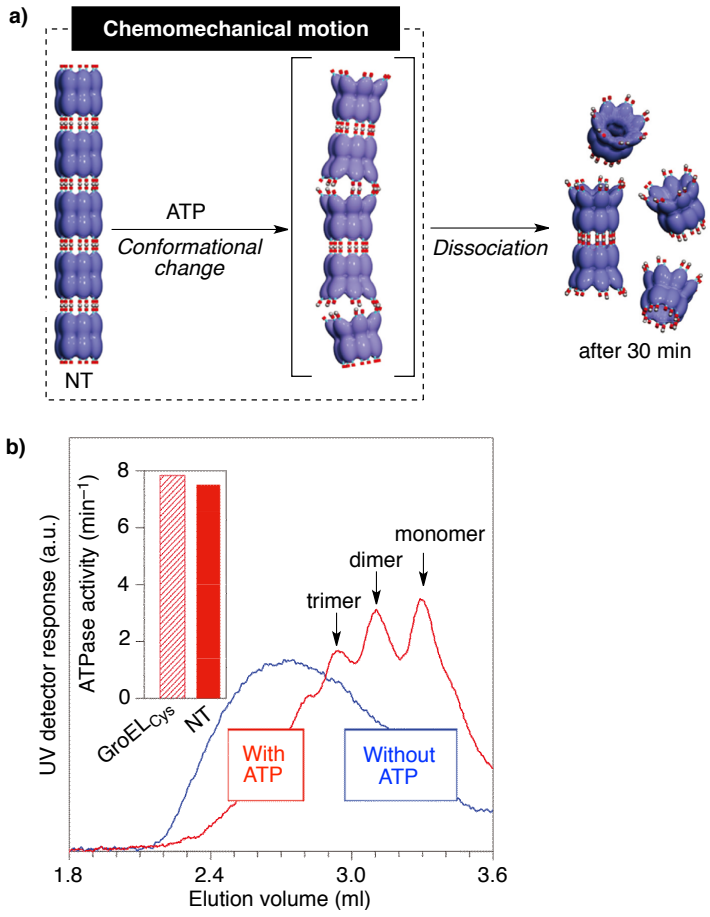

Figure 4. Dissociation of $GroEL_{MC}$ nanotubes. (a) Schematic image of he dissociation. (b) SEC spectra of the nanotubes.

• In TEM observation, in the case of $GroEL_{MC}$ nanotubes (SH/MC-conversion = 82%), it was observed that the nanotubes were dissociated into smaller oligomers or monomer than $GroEL_{MC}$ nanotubes (SH/MC-conversion = 100%) due to the less -amount of MC–Mg^{2+}–MC bonds (Figure 5).

• In the case of $GroEL_{MC}$ nanotubes (SH/MC-conversion = 100%), treatment with ATP causes nanotubes to become heavily twisted and wavy (Figure 5b).

=> The chemomechanical motions of the repeating $GroEL_{MC}$ units give rise to a large strain in the nanotubes.

=> A mechanical force generated by a single conformational change of $GroEL_{MC}$ is large enough to cut the multiple MC–Mg^{2+}–MC bonds in a nanotube.

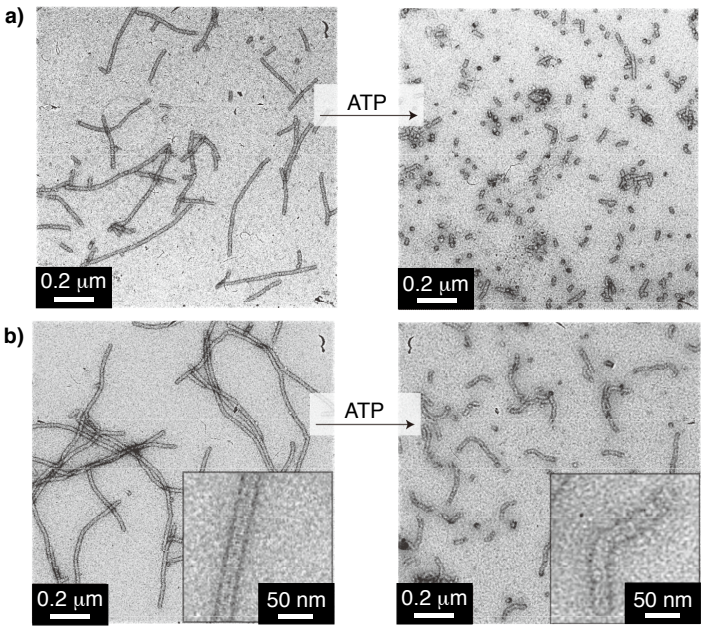

Figure 5. TEM images of $GroEL_{MC}$ nanotubes before (left) and after (right) incubation with ATP. (a) $GroEL_{MC}$ (SH/MC-conversion = 82%). (b) $GroEL_{MC}$ (SH/MC-conversion = 100%).

2.2. Preparation of cell-penetrable dye-containing nanotubes

• $GroEL_{MC}$ nanotubes are difficult to enter cells because of weak adhesion to the cell membrane.

• 2-(hydroxymethyl)phenylboronic acid cyclic monoester (BA) derivative can bind preferentially to the terminal positions of carbohydrate chains of glycoproteins and glycolipids in cell membrane.[3]

• Amine moieties on the surface of the nanotubes were modified with the BA derivative (Figure 6a).

=> The resulting boronic acid-functionalized nanotube (^{BA}NT) was indeed able to penetrate cells.

• Denatured α-lactalbumin (α-LA_{denat}) is non-releasable protein from cavity of GroEL.[4]

=> A stable scaffold for anchoring dyes (Figure 6b)

• Dye molecule was anchored via ester bond.

=> Cleavage of the ester bond by intracellular esterase induces release of dye from the cavity.

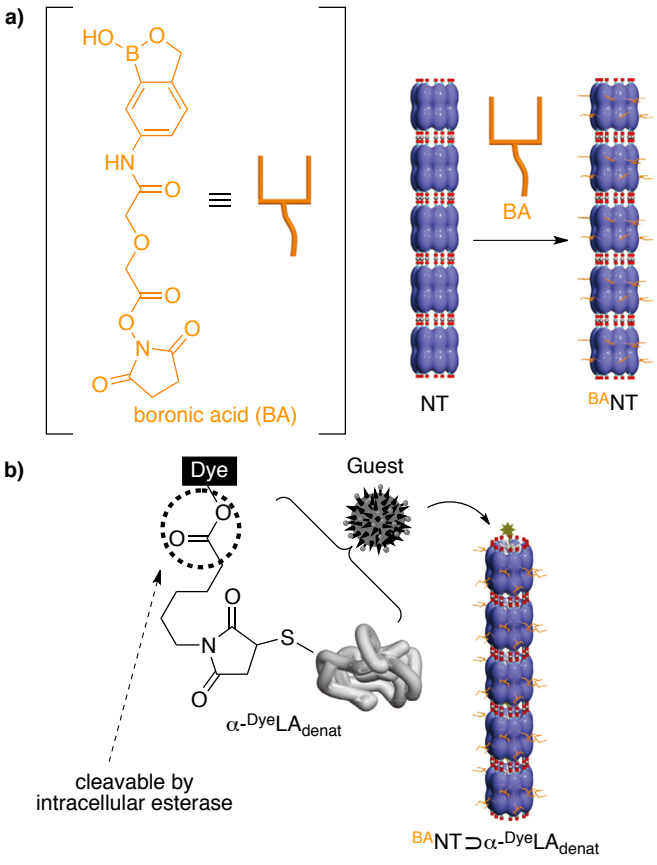

Figure 6. Cell-penetrable dye-containing nanotube. (a) Schematic illustration of the preparation of cell-penetrable ^{BA}NT. (b) Design of dye-containing guest.

2.3. ATP-responsive intracellular delivery

• Concentration of intracellular ATP (1–10 mM) is enough to dissociate $GroEL_{MC}$ nanotubes but that of extracellular ATP (< 5 μM) is not enough (Figure 7a).

• Dye molecules are released from ^{BA}NT by ATP-induced dissociation of the nanotube and cleavage of the ester bond of α-LA_{denat} by esterase after uptake into Hera cell (Figure 7b).

• Cyanine dye is quenched when it is anchored to α-LA_{denat} via ester bond, but after cleavage of the bond, the dye emits light (at λ_{em} = 611 nm).

• After incubation with ATP (200 μM) for 60 min, the fluorescence of cyanine dye was observed in the presence of esterase. Unless esterase was present, incubation with ATP did not give rise to the fluorescence (Figure 7c).

• By confocal laser scanning fluorescence micrograph of Hera cells after incubation with $^{BA}NT \supset \alpha\text{-}^{Dye}LA_{denat}$ for 16 h, the fluorescence of the dye was observed in Hera cell.

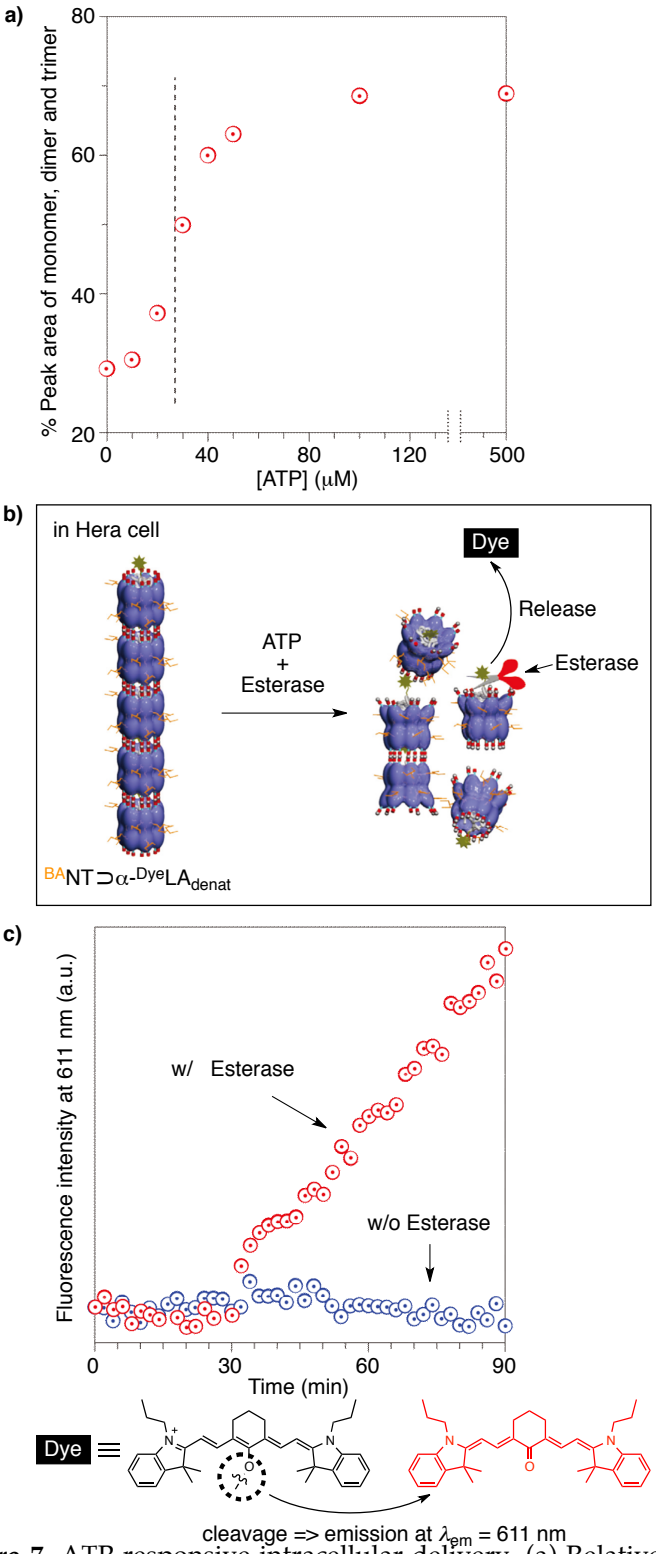

Figure 7. ATP-responsive intracellular delivery. (a) Relative SEC-UV peak areas (%) of the fraction of short-chain oligomers after incubation of NT. (b) Schematic illustration of the basic strategy of ATP-responsive intracellular delivery. (c) Time courses of the fluorescence intensity change at λ_{em} = 611 nm.

3. Conclusion

• ATP-induced dissociation of $GroEL_{MC}$ nanotubes was demonstrated.

• The dissociation is driven by a mechanical force generated by a single conformational change of $GroEL_{MC}$.

• Post-modification of the nanotubes with boronic acid derivative functionalized them to penetrate cell membrane.

• ATP-responsive intracellular delivery of cyanine dye anchored to denatured α-lactalbumin via enzymatically cleavable linker was demonstrated.

[1] Hartl, F. U.; Bracher, A.; Hayer–Hartl, M. *Nature* **2011**, *475*, 324–332.
[2] Biswas, S.; Kinbara, K.; Oya, N.; Ishii, N.; Taguchi, H.; Aida, T. *J. Am. Chem. Soc.* **2009**, *131*, 7556–7557.
[3] Ellis, G. A.; Palte, M. J.; Raines, R. T. *J. Am. Chem. Soc.* **2012**, *134*, 3631–3634.
[4] Shimizu, A.; Tanba, T.; Ogata, I.; Ikeguchi, M.; Sugai, S. *J. Biochem.* **1998**, *124*, 319–325.