Reconstitution of microtubule into GTP-responsive nanocapsules

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Nanocapsules that collapse in response to guanosine triphosphate (GTP) have the potential as drug carriers for efficiently curing diseases caused by cancer and RNA viruses because GTP is present at high levels in such diseased cells and tissues. However, known GTP-responsive carriers also respond to adenosine triphosphate (ATP), which is abundant in normal cells as well. Here, we report the elaborate reconstitution of microtubule into a nanocapsule that selectively responds to GTP. When the tubulin monomer from microtubule is incubated at 37 °C with a mixture of GTP (17 mol%) and nonhydrolysable GTP* (83 mol%), a tubulin nanosheet forms. Upon addition of photoreactive molecular glue to the resulting dispersion, the nanosheet is transformed into a nanocapsule. Cell death results when a doxorubicin-containing nanocapsule, after photochemically crosslinked for properly stabilizing its shell, is taken up into cancer cells that overexpress GTP.

An ideal nanocarrier for drug delivery would be the one that can selectively collapse to release preloaded drugs in response to endogenous reporters overexpressed in disease tissues1-9. Since adenosine triphosphate (ATP) is known to be present at high levels in cancer tissues10, ATP-responsive nanocarriers might be a promising candidate3-7. In 2013, using partially modified biomolecular machine chaperonin GroEL as a monomer, we succeeded in developing a one-dimensional supramolecular polymer that can be depolymerized by the action of ATP to release its cargo1. However, ATP is also present in normal cells at rather high concentrations (>1 mM)11, and thus selective drug delivery using ATP as the endogenous reporter cannot always be ensured. In the present work, we developed a nanocarrier (NC\textsubscript{GTP/GTP*}; Fig. 1e) that selectively responds to guanosine triphosphate (GTP). GTP is an intracellular molecule involved in many essential biological processes12-31, such as cell division12, nucleotide synthesis13, and cell signaling14. In the cell division process, the tubulin heterodimer (THD), which constitutes microtubules (MTs), uses GTP as an energy source to induce its polymerization and depolymerization15-20. GTP is also used as a component for the self-replication of RNA viruses26-29 such as coronaviruses. Notably, GTP is abundant in certain diseased cells (1.5-4.5 mM)30 such as rapidly proliferating cancer cells31 and RNA virus-infected cells32, whereas the concentration of GTP, unlike that of ATP, is negligibly low in normal cells (<0.3 mM)13. Therefore, GTP-responsive nanocarriers have the great potential to efficiently cure cancer and RNA virus-induced diseases including coronavirus disease 2019 (COVID-19)29. Although GTP-responsive carriers have already been reported, those carriers also respond to ATP5. So far, nanocarriers capable of responding solely to GTP have never been reported.

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The nanocapsule (NC) that selectively responds to GTP to release a preloaded drug consists of THD. As shown in Fig. 1a, THD is composed of α-tubulin (green) and β-tubulin (cream), both of which bind to GTP. Notably, GTP attached to the α-tubulin unit is neither hydrolysable into GDP nor replaceable with other nucleoside phosphates. In contrast, GTP attached to the β-tubulin unit is known to be hydrolysable to GDP, which can be replaced with, e.g., GTP*, a nonhydrolysable GTP analogue (guanylyl 5'-α,β-methylene diphosphate), affording THD_{GTP} (for convenience, only variable nucleoside phosphates attached to the β-tubulin unit are shown as a cliche.

**Fig. 1** | Strategy used to prepare THD-based GTP-responsive NC_{GTP/GTP*}.

- **a** Schematic illustrations of tubulin heterodimers (THDs) hybridized with GTP (THD_{GTP}), its nonhydrolysable analogue GTP* (THD_{GTP*}), and GDP (THD_{GDP}) at its β-tubulin unit.
- **b** Schematic illustration of two self-assembling modes of THD into microtubules (MTs). MT_{GTP} depolymerizes into THD_{GDP} upon GTP hydrolysis.
- **c** Tubulin heterodimers (THDs) hybridized with nucleoside phosphates.
- **d** Exchange reaction between GDP and GTP.
- **e** Schematic illustration of the multistep procedure for the synthesis of crosslinked nanocapsules (NC_{GTP/GTP*}) from MT_{GTP}. MT_{GTP} is depolymerized into THD_{GDP}, which is incubated with a mixture of GTP* (83 mol%) and GTP (17 mol%) to form nanosheet NS_{GTP/GTP*}. Upon treatment with GlueCO2/C0, NS_{GTP/GTP*} is transformed into spherical nanocapsules (NC_{GTP/GTP*}), which are further exposed to UV light, affording NC_{GTP/GTP*}. Upon addition of GTP, NC_{GTP/GTP*} collapses through the conformational change of the THD units induced by GTP hydrolysis.
Both THDGTP and THDGTP*, when heated at 37 °C, have been reported to self-assemble into microtubules MTGTP and MTGTP*, respectively (Fig. 1b). Although MTGTP depolymerizes into THDGDP synchronously with the hydrolysis of hybridized GTP to GDP, THDGTP* does not depolymerize into THDGDP* because of the non-hydrolysable nature of GTP*. Therefore, our original motivation was to tackle a challenge of modulating the stability of MTs against depolymerization by changing the THDGTP/THDGTP* molar ratio. However, we unexpectedly found that the coassembly of THDGTP and THDGTP* at a certain mixing molar ratio resulted in the formation of a leaf-like 2D nanosheet (NS) rather than MT (Fig. 1e). Because of the increasing importance of 2D objects, this finding prompted us to functionalize NS using the molecular glue technology \[^{35-36}\] which we developed for noncovalently functionalizing biomolecules such as proteins, nucleic acids, and phospholipid membranes, and also inorganic materials. Molecular glues are designed to carry multiple guanidinium ion (Gu\(^+\)) groups and strongly adhere to such biomolecules under physiological conditions by taking advantage of a multivalent salt-bridge interaction with their oxyanionic functionalities (Fig. 1d). For this purpose, we chose Glue\(_{CO2}\) (Fig. 1c) and incubated it with NS. To our surprise, NS was transformed into a spherical nanocapsule NC (Fig. 1e). Using its photochemically modified version (\(^{36}\)NCGTP/GTP*; Fig. 1e), we successfully encapsulated and delivered doxorubicin (DOX)\(^{4}\), an anticancer drug, into GTP-overexpressing cancer cells to cause cell death.

**Results**

**Reconstitution of MTGTP into NCGTP/GTP**

Figure 1e illustrates the overall procedure for the synthesis of NCGTP/GTP from microtubule MTGTP. As a typical example of the procedure depicted in the flow chart in Fig. 2a, a 1,4-piperazinediethanesulfonic acid (PIPES) buffer (pH 6.8) solution of MTGTP (5.8 mg ml\(^{-1}\)) was cooled at 4 °C, whereupon MTGTP underwent complete depolymerization within 3 h to yield THDGDP quantitatively (Fig. 2d). As observed by dynamic light scattering (DLS), the characteristic polydisperse feature of one-dimensional (1D) MTGTP (Fig. 2b, gray) changed to a monodisperse feature with a reduced hydrodynamic diameter of 8 nm (Fig. 2b, blue). Then, THDGDP (0.3 mg ml\(^{-1}\)) was immersed in a PIPES buffer solution of a mixture of GTP and GTP* (300 \(\mu\)M in total) with a GTP* content of 83 mol% at 37 °C for 30 min. Under the present conditions, THDGDP was converted via the exchange events of GDP with a GTP* content of 83 mol% at 37 °C for 30 min. Under the present conditions, THDGDP was critical for its successful transformation into NSGTP/GTP*.

**Photochemical crosslinking of NCGTP/GTP**

The physical stability of NCGTP/GTP is important for its utilization as a carrier for drug delivery. Through several different experiments, we noticed that NCGTP/GTP immediately collapsed upon incubation with albumin or serum in buffer, indicating its insufficient stability as a drug carrier. Here, we would like to point out a great advantage of Glue\(^{CO2}\) and its homologues that their multiple benzenophenone (BP) groups upon photoexcitation enable covalent crosslinking with adhering proteins (Fig. 1d). Successful examples so far reported include microtubule and kinesin\(^{37}\), whose dynamic behaviors could be attenuated by the reaction with photoexcited molecular glues. In the present work, by using fluorescent FITC-appended Glue\(_{CO2}\) (Fig. 1c, FITC; fluorescein isothiocyanate) derived from Glue\(^{CO2}\), we first confirmed that Glue\(^{CO2}\) has a sufficient photoreactivity with the constituent (THD) of NCGTP/GTP. As shown in Supplementary Fig. 17, the reaction mixture, after being exposed to UV light (300 nm) in PIPES buffer, showed the presence of a fluorescence-emissive covalent adduct between THDGDP and Glue\(^{CO2}\) in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Then, we investigated whether this photochemical approach can provide NCGTP/GTP* with a sufficient physical stability by crosslinking the shell. Thus, a PIPES buffer solution of NCGTP/GTP* was exposed to UV light for 2 min, where TEM (Fig. 2h) and AFM imaging results (Supplementary Fig. 18) and DLS profiles (Fig. 2b, red) showed that crosslinked (CL) NCGTP/GTP* was spherical and remained intact even upon incubation with albumin (0.1 mg ml\(^{-1}\)) or serum (0.01%) (Supplementary Figs. 19 and 20). NCGTP/GTP* when prepared using Glue\(^{CO2}\) instead of Glue\(^{CO2}\) was fluorescent (Supplementary Fig. 21), indicating the presence of the molecular glue in NCGTP/GTP*.

**Computational simulation of the assembly of NSGTP/GTP**

Considering that tubulin nanosheets NCGTP/GTP are, on average, 0.44 µm\(^2\) wide and 4.2 nm thick, the formation of NCGTP/GTP (surface area; 6.2 µm\(^2\), membrane thickness; 50 nm) requires at least 1000 pieces of NSGTP/GTP to assemble. Note that Glue\(^{CO2}\) carrying both Gu\(^+\) and CO\(_2\) –groups in its structure can self-assemble via their salt-bridge interaction. In the initial stage of the transformation of NCGTP/GTP into NCGTP/GTP*, we postulate that a certain number of Glue\(^{CO2}\) molecules utilize their Gu\(^+\) groups to form a salt-bridged network with the surface CO\(_2\) –groups on NCGTP/GTP* (Fig. 1d) as well as the focal-core CO\(_2\) group in Glue\(^{CO2}\). This adhesion event can lower the surface charge density of NCGTP/GTP* and enhance its hydrophobic stacking, which is secured by possible reorganization of the salt-bridged polymeric networks on NCGTP/GTP* (Fig. 1e). We performed all atom molecular dynamics (MD) simulations \[^{39}\] to explore the adhesion of Glue\(^{CO2}\) and the effect of this event on the tubulin assembly. From a full MT model (PDB code: 3J6E), we obtained its partial structure composed of three laterally assembled THDGTP units (THDGTP\(_3\)) as a model of NS (Fig. 3a). The MD simulation suggested that Glue\(^{CO2}\) adopts a globular conformation in aqueous media with a hydrodynamic diameter of 5.0-10.0 nm (Fig. 3b).
1.5 nm (Fig. 3b, Supplementary Fig. 22). When exposed to 30 equivalents of GlueCO2/C0 (Fig. 3c, d), [THDGTP*]3 enhances its hydrophobic nature (Fig. 3e, f) as a result of the surface charge neutralization by adhering GlueCO2/C0. In the solvent-accessible surface area of [THDGTP*]3, the hydrophobic dominancy increases from 48% to 57% (Fig. 3g). Notably, when GlueCO2/C0 was allowed to adhere onto [THDGTP*], the molecular simulations suggested that [THDGTP*]3 adopts a slightly flattened conformation, characterized by a distribution angle with an average value of ~156° (Fig. 3h, i, blue), compared with that of the native [THDGTP*]3 (red). The simulations also showed that, even after the GlueCO2/C0 adhesion, [THDGTP*]3 preserved a certain level of flexibility (Fig. 3i). We also calculated radial distribution functions g(r) between the charged groups of GlueCO2/C0 and the amino acid residues of [THDGTP*]. Supposedly, the CO2– groups in aspartic acid and glutamic acid are interactive with the Gu+ groups in GlueCO2/C0, while the cationic groups in lysine and arginine are interactive with the focal CO2– group in GlueCO2/C0. As expected, the g(r) data revealed that the Gu+ groups in GlueCO2/C0 are largely populated near the CO2– groups on [THDGTP*]3. 

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**Fig. 2 | Reconstitution of MT into CLNCGTP/GTP*.** a A typical synthetic procedure for the preparation of CLNCGTP/GTP*. b DLS profiles of MTGTP (gray), THDGDP (blue), NSGTP/GTP* (green), NCGTP/GTP* (orange), and CLNCGTP/GTP* (red) in PIPES buffer. c–e TEM images of MTGTP (5.8 mg ml⁻¹; c), THDGDP (0.3 mg ml⁻¹; d), and NSGTP/GTP* (0.3 mg ml⁻¹; e). f AFM image of NSGTP/GTP* (0.3 mg ml⁻¹) and its height profile. g, h TEM images of NCGTP/GTP* (13 µg ml⁻¹; g) and CLNCGTP/GTP* (13 µg ml⁻¹; h). All TEM samples were negatively stained with uranyl acetate. Inset scale bars, 250 nm. 

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Fig. 3 | MD simulation of the adhesion events of GlueCO2 onto the surface of THDGTP.* a Three laterally assembled THDGTP* units ([THDGTP*]3) in MTGTP as a partial model of NS. b An equilibrated MD snapshot of GlueCO2. c, d The outer (c) and inner (d) views of [THDGTP*], hybridized with 30 equivalents of GlueCO2. e, f The outer (e) and inner (f) views of [THDGTP*], with its electrostatic surface potential in the absence (upper) and presence (lower) of 30 equivalents of hybridized GlueCO2. Negative and positive potential areas are colored in red and blue, respectively. g The percentage of hydrophobic solvent-accessible surface area in the absence (47.5 ± 0.5; red) and presence (56.7 ± 2.0; blue) of 30 equivalents of hybridized GlueCO2. Bars represent mean values ± SD from 2000 data points. h, i [THDGTP*] observed from the top view (h) and its angle distributions (i) in the absence (red) and presence (blue) of 30 equivalents of hybridized GlueCO2. j Radial distribution functions g(r) of the Gu+ groups in GlueCO2 with carboxylates (blue) and non-ionic hydroxyl groups (gray) on the [THDGTP*] surface, and the carboxylate at the focal core of GlueCO2 (red). k Schematic illustration of a possible adhesion event of GlueCO2 onto NSGTP/GTP* and its effects on the features of NSGTP/GTP*. The Gu+ groups in GlueCO2 form a salt bridge with carboxylates on the NSGTP/GTP* surface and at the focal core of GlueCO2, and the GlueCO2-based polymeric network thus formed through this process increases the hydrophobicity of the NSGTP/GTP* surface, making NSGTP/GTP* more flatter.
the [THDGTP]3 surface (Fig. 3j, blue), whereas they are scarcely populated around the polar but nonionic hydroxyl groups in serine, threonine, and tyrosine (Fig. 3j, gray). Meanwhile, the computational calculation also showed that multiple adhering GlueCO2+ molecules can interact and self-assemble via an electrostatic bridging interaction between their Cu+ and CO2– groups (Fig. 3j, red), which results in forming a dense Gu+–CO2– salt-bridged polymeric network on the [THDGTP]3 surface (Fig. 3k). This may promote the self-assembly of flexible NSGTP/GTP* and stabilize them in the gently curved multilayered configuration of NC/GTP, as observed experimentally.44,45. As a control experiment, the use of GlueCO2–Me (Fig. 1c) having a focal ester group instead of its ionized form for the transformation of NSGTP/GTP* into NGTP/GTP* resulted in an ill-defined agglomerate (Supplementary Fig. 24).

**GTP-responsiveness of** $[^{13}]$NC/GTP/GTP*

We investigated whether photochemically stabilized $[^{13}]$NC/GTP/GTP* is responsive to GTP or not. Notably, the concentrations of both extra- and intracellular GTP are lower than 0.3 mM in normal cells33. However, as already described in the introductory part, rapidly proliferating cancer cells and RNA virus-infected cells contain GTP in a concentration range of 1.5–4.5 mM.34 Therefore, drug-loaded $[^{13}]$NC/GTP/GTP*, when taken up into such GTP-rich environments, might selectively collapse to release its preloaded guest. Upon incubation for 100 min at 37 °C in PIPES buffer with 0.2 mM GTP, $[^{13}]$NC/GTP/GTP* still maintained its spherical shape, as observed by TEM (Fig. 4a). However, when the GTP concentration was increased to 0.5 mM, $[^{13}]$NC/GTP/GTP* gradually collapsed (Fig. 4b), displaying a polydisperse DLS profile in 100 min (Fig. 4c, green). This minimum concentration threshold is important for achieving the error-free delivery to GTP-enriched sites. We added Bimol Green as a phosphoric acid (PO43-) detector to a mixture of $[^{13}]$NC/GTP/GTP* and GTP (1 mM), and successfully detected PO43- by means of electronic absorption spectroscopy, indicating that $[^{13}]$NC/GTP/GTP* has a GTPase activity (Fig. 4d). Although THDGTP*, the constituent of $[^{13}]$NC/GTP/GTP*, has no GTPase activity, the product upon incubation of THDGTP* with GTP, DOX was also successful with other cell lines such as A549 cell and HeLa cell (Supplementary Fig. 35). We also confirmed that Hep3B cells took up a larger amount of DOX in $[^{13}]$NC/GTP/GTP* (Fig. 5f, red) than DOX alone (Fig. 5f, orange). Accordingly, $[^{13}]$NC/GTP/GTP*–DOX successfully lowered the cell viability to 30 ± 6% (Fig. 5g, red), whereas that caused by DOX alone was only 48 ± 15% (Fig. 5g, orange). As expected, the cell viability decreased as the concentration of $[^{13}]$NC/GTP/GTP*–DOX was increased (Supplementary Fig. 33), while the viability upon incubation with $[^{13}]$NC/GTP/GTP–DOX did not substantially increase when the incubation time was shortened from 2.5 h to 0.5 h (Supplementary Fig. 34). This is likely caused by the GTP-selective collapse of $[^{13}]$NC/GTP/GTP*. The intracellular delivery of $[^{13}]$NC/GTP/GTP–DOX was also successful with other cell lines such as A549 cell and HeLa cell (Supplementary Fig. 35). We also confirmed that neither the coexistence of THDGTP* nor THDGTP*–GlueCO2– enhanced the efficacy of DOX (Supplementary Fig. 36). Together with the noncytotoxic nature of $[^{13}]$NC/GTP/GTP* (Fig. 5g, green) and its stability in a range of pH at tumor tissue (Fig. 2b), these results allow us to expect that $[^{13}]$NC/GTP/GTP* may have the potential to deliver preloaded drugs into cancer cells using GTP as an endogenous reporter.

**Discussion**

Here, we have documented the successful reconstitution of MT/GTP into a GTP-responsive nanocarrier (Fig. 1). MT/GTP is depolymerized into THDGTP*, which is incubated with a mixture of GTP* and GTP (content of GTP*: 70–85 mol%), thereby facilitating the in situ coassembly of the resulting THDGTP* and THDGTP* monomers to form NSGTP/GTP*. Subsequently, NSGTP/GTP* is treated with molecular glue $[^{13}]$NC/GTP/GTP* to be transformed into spherical NSGTP/GTP*, followed by UV exposure to afford crosslinked $[^{13}]$NC/GTP/GTP* capable of stably
encapsulating guests (Fig. 2). In GTP-rich environments, CLNCGTP/GTP* collapses and releases preloaded guests through the transformation of CLNCGTP/GTP* into CLNCGTP followed by the hydrolysis of its bound GTP into GDP, analogous to the depolymerization of MTGTP (Fig. 4). Using CLNCGTP/GTP*, we successfully delivered DOX into cancer cells that overexpress GTP, and caused cell death more efficiently than DOX alone (Fig. 5). Most importantly, CLNCGTP/GTP* is a drug carrier that can selectively collapse in response to GTP rather than ATP that is abundant in normal cells. Since cells infected with RNA viruses such as coronavirus produce a large amount of GTP in their self-replication process, GTP is an endogenous reporter for RNA virus-infected cells. In vivo utilization of CLNCGTP/GTP* for curing RNA virus-induced diseases such as COVID-19 is one of the interesting subjects worthy of further investigation.

**Methods**

MD simulation was performed using AmberTools 20, GROMACS 2020.5 package, and Visual Molecular Dynamics (VMD) package, and MD simulation methodologies are described in the Supplementary Information.

**Reconstitution of MTGTP into CLNCGTP/GTP**

THDGTP was obtained by purification from porcine brain by two cycles of polymerization and depolymerization in PIPES buffer.

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**Fig. 4 | GTP-triggered collapse of CLNCGTP/GTP.** a, b TEM images of CLNCGTP/GTP after a 100-min incubation with GTP at its concentrations of 0.2 mM (a) and 0.5 mM (b). c DLS profiles of CLNCGTP/GTP (8.7 µg ml⁻¹) in PIPES buffer after a 100-min incubation with GTP at its concentrations of 0 mM (red), 0.2 mM (orange), 0.5 mM (green), and 1 mM (blue). d GTPase activities of THDGTP (left) and CLNCGTP/GTP (right) in PIPES buffer. The data was obtained from three biologically independent samples (n = 3). e DLS profiles of CLNCGTP/GTP (8.7 µg ml⁻¹) in PIPES buffer after a 100-min incubation with 1 mM of ATP (red), CTP (orange), and UTP (green). f TEM image of CLNCGTP/GTP ⊂ NPAu ([CLNCGTP/GTP] = 13 µg ml⁻¹, [NPAu] = 13 pM). g CLSM images of FITC-labeled CLNCGTP/GTP ⊂ DOX ([CLNCGTP/GTP] = 13 µg ml⁻¹, [DOX] = 10 µM) incubated without (upper panel) and with (lower panel) 1 mM GTP at 37 °C for 100 min. Micrographs display locations of FITC (i, green) and DOX (ii, red), and their merged images (iii). Scale bars, 2.0 µm. h Fluorescence intensities at 590 nm (λext = 470 nm) of residual DOX obtained after 20, 50, and 100-min incubations of a PIPES solution of CLNCGTP/GTP ⊂ DOX with 1 mM GTP, followed by ultrafiltration. Red bars represent mean values ± SD from three different samples.
**Fig. 5 | Intracellular drug delivery using \(^{14}\text{C}\text{GTP}\text{GTP}^*\).** a Schematic illustration of the uptake of FITC-labeled \(^{14}\text{C}\text{GTP}\text{GTP}^*\) into Hep3B cells. b Bright field (upper row) and CLSM images displaying FITC (middle row, green) in Hep3B cells and their merged images (lower row). The cells were incubated in EMEM containing \(^{14}\text{C}\text{GTP}\text{GTP}^*\) (0.5 \(\mu\text{g ml}^{-1}\)) for 2.5 h, rinsed with D-PBS, and further incubated in EMEM (10% FBS) for 1.5 h (i) and 21.5 h (ii). Scale bars, 20 \(\mu\text{m}\). c Flow cytometry profiles showing FITC fluorescence of Hep3B cells (n > 660) incubated without (blue) and with FITC-labeled \(^{14}\text{C}\text{GTP}\text{GTP}^*\) for 2.5 h, rinsed with D-PBS, and further incubated in EMEM (10% FBS) for 1.5 h (i, orange) and 21.5 h (ii, green). d Schematic illustration of the cellular uptake of \(^{14}\text{C}\text{GTP}\text{GTP}^*\)-DOX. e Bright field (upper row) and CLSM images displaying DOX (middle row, red) in Hep3B cells and their merged images (lower row). The cells were incubated in EMEM containing \(^{14}\text{C}\text{GTP}\text{GTP}^*\)-DOX \(([^{14}\text{C}\text{GTP}\text{GTP}^*\text{-DOX}] = 2.6 \mu\text{g ml}^{-1}, [\text{DOX}] = 2 \mu\text{M})\) for 2.5 h, rinsed with D-PBS, and further incubated in EMEM (10% FBS) for 1.5 h (iii) and 21.5 h (iv). Scale bars, 20 \(\mu\text{m}\). f, g Flow cytometry profiles (f) showing DOX fluorescence of Hep3B cells (n = 390) and their normalized viabilities (g) determined using Cell Counting Kit-8 (n = 3). The cells were incubated without (blue) and with DOX (2 \(\mu\text{M}\); orange), and \(^{14}\text{C}\text{GTP}\text{GTP}^*\)-DOX \(([^{14}\text{C}\text{GTP}\text{GTP}^*\text{-DOX}] = 2.6 \mu\text{g ml}^{-1}, [\text{DOX}] = 2 \mu\text{M}; \text{red})\) for 2.5 h in EMEM, and then rinsed with D-PBS, followed by incubation in EMEM (10% FBS) for 21.5 h. Statistical significance was examined by two-sided Student’s t-test \((p = 0.0094 < 0.01)\). Bars represent mean values ± SD from three different samples.
(100 mM PIPES, 2 mM MgSO₄, 0.5 mM GTP, 4 µg ml⁻¹ leupeptin, and 0.4 mM PefABlock, pH 6.8). A solution of THDGDP (5.8 mg ml⁻¹) in PIPES buffer (100 mM PIPES, 5 mM MgCl₂, 2 mM MgSO₄, 1.5 mM GTP, and 10% DMSO, pH 6.8) was incubated at 37 °C for 30 min to afford MTGTP. The reaction mixture was centrifuged at 17,900 × g for 20 min at 24 °C. The resulting precipitate was dissolved in PIPES buffer (100 mM PIPES, 100 mM MgCl₂, and 200 µM GDP, pH 6.8) and incubated at 4 °C for 3 h to afford THDGDP. Subsequently, THDGDP (0.3 mg ml⁻¹) thus obtained was incubated in PIPES buffer (100 mM PIPES, 1 mM MgCl₂, 250 µM GTP*, and 50 µM GTP, pH 6.8) at 4 °C for 60 min and then at 37 °C for 30 min to afford NSPGDP. NSPGDP (13 µg ml⁻¹) was incubated in a solution of Glue⁻CO₂⁻ (100 µM) in PIPES buffer (14 mM PIPES, 1 mM MgCl₂, and 200 µM GTP, pH 6.8) at 37 °C for 30 min. The reaction mixture was exposed to UV light at 300 nm for 2 min, affording CNGTP/GTP*. FITC-labeled NSPGDP- and CNGTP/GTP* were prepared using FITC-labeled THDGDP (14% labeling rate) under conditions that were otherwise identical to those listed above. Prior to the NMR measurement of the CNGTP/GTP* sample, unbound GTP and GTP* were removed by centrifugation (286,000 × g) of the reaction mixture at 37 °C for 60 min. Zeta potentials of CNGTP/GTP* (1.3 µg ml⁻¹) and NCNGTP/GTP* (1.3 µg ml⁻¹) were measured at 37 °C in PIPES buffer.

**Preparation of CNGTP/GTP*→NP Au**

CNGTP/GTP*→NP Au was prepared after the incubation of a mixture of CNGTP/GTP* (13 µg ml⁻¹), Glue⁻CO₂⁻ (100 µM), and gold nanoparticles (NP Au, 14 µM) in PIPES buffer (14 mM PIPES, 1 mM MgCl₂, and 200 µM GTP, pH 6.8) at 37 °C for 30 min, followed by UV irradiation at 300 nm for 2 min. For the asymmetric field flow fractionation analysis, a sample solution of CNGTP/GTP*→NP Au in PIPES buffer was subjected to ultrafiltration (1500 × g) for 5 min using a regenerated cellulose membrane (cut-off MW = 50000) prior to analysis. PEG-coated NP Au was used to avoid nonspecific adhesion of THD²⁻.

**GTP-triggered release of DOX from CNGTP/GTP**

CNGTP/GTP*→DOX was prepared after the incubation of a mixture of CNGTP/GTP* (13 µg ml⁻¹), Glue⁻CO₂⁻ (100 µM), and DOX (10 µM) in PIPES buffer (14 mM PIPES, 1 mM MgCl₂, and 400 µM GTP, pH 6.8) at 37 °C for 30 min, followed by UV irradiation at 300 nm for 2 min. The reaction mixture was incubated with GTP (1 mM) at 37 °C for 100 min and then subjected to ultrafiltration (2400 × g) using a regenerated cellulose membrane (cut-off MW = 5000) for 10 min. The resulting residue was subjected to fluorescence spectroscopy (λ_{ex} = 470 nm). A reference sample without GTP was likewise prepared.

**Intracellular delivery**

Hep3B cells (3.0 × 10⁷ cells/well) plated onto an 8-well chambered cover glass were incubated in EMEM containing 10% FBS at 37 °C with 5% CO₂ for 24 h. The cell samples were rinsed twice with D-PBS prior to use. Typically, the cells were treated with FITC-labeled CNGTP/GTP* (0.5 µg ml⁻¹) and incubated at 37 °C with 5% CO₂ for 2.5 h. Then, the cells were rinsed twice with D-PBS and further incubated at 37 °C for 1.5 h (4-h incubation in total) or 21.5 h (24-h incubation in total) with 5% CO₂ in EMEM containing 10% FBS. Analogous cell samples treated with FITC-labeled THDGDP (0.5 µg ml⁻¹), FITC-labeled NSPGDP (0.5 µg ml⁻¹), CNGTP/GTP* (0.5 µg ml⁻¹) with NaN₃ (5 mM)⁷,¹, CNGTP/GTP*→DOX ([¹⁴C]GTP/GTP*) = 2.6 µg ml⁻¹. [DOX] = 2 µM, CNGTP/GTP* (2.6 µg ml⁻¹), and DOX (2 µM) were likewise prepared. For a cell viability assay using CNGTP/GTP*→DOX, CNGTP/GTP* and DOX, the cell samples were treated with Cell Counting Kit-8 reagents (10 µl) for 30 min, and subjected to electronic absorption spectroscopy at 450 nm. Hep3B cell samples treated with TWEEN 20 (0.2%) were used as a positive control.

**Statistics and reproducibility**

All experiments including the preparation of CNGTP/GTP*, the investigation of its GTP-responsive collapse, and the intracellular delivery using CNGTP/GTP* were performed at least three times to check the reproducibility.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All the data corresponding to the findings of this study are provided in the article and Supplementary Information. Source data is available for Figs. 2b, 3g, i, j, 4c–e, h, c, f, and g and Supplementary Figs. 9–11, 13–16, 19–25, 27, 28 and 30–36 in the associated source data file. 3D structures of THD for the MD simulation were obtained from Protein Data Bank (PDB) (PDB code: 3J6E and 1TUB). Complete modeling data, structures and parameters used for, and extracted from simulations are available at https://zenodo.org/record/7070651#.Yx80t9JBxkg. Source data are provided with this paper.

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Author contributions
N.U., A.K., and K.O. designed and performed all experiments; A.C., C.L., E.A.Z., M.D., and G.M.P. performed and analyzed the MD simulation; N.U. and M.T. prepared THD; T.H. supported the small-angle X-ray scattering measurements at SPring-8; N.U., G.M.P., and T.A. analyzed the data and wrote the manuscript.

Competing interests
The authors declare no competing interests.
