Supplementary Information (SI) to accompany

**pH responsive ATP carriers to drive kinesin movement**

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Experimental section

Materials:

Unlabeled tubulin, biotinylated tubulin and rhodaminelabeled tubulin, Paclitaxel were purchased from Cytoskeleton Company. Sodium carbonate (Na$_2$CO$_3$), calciumchloride (CaCl$_2$), adenosine triphosphate (ATP), glucose oxidase (GOD), catalase (CAT), casein, β-D-Glucose (G) and poly(allylamine hydrochloride) (PAH), Poly(sodium-p-styrenesulfonate) (PSS) were obtained from Sigma-Aldrich. All chemicals were used without further purification.

The water used in all experiments was prepared in a three-stage Millipore Milli-Q plus 185-purification system and had a resistivity higher than 18.2 MΩ.

Kiensin and microtubules:

Full length kinesin-1 was expressed and purified as described elsewhere$^{1,2}$.

Rhodamine-labeled microtubules were copolymerized with biotinylated tubulin, rhodamine modified tubulin and the unlabeled tubulin at the ratio of 1:1:1. These tubulins were copolymerized with a final concentration of 4 mg mL$^{-1}$ (Cytoskeleton, Denver, CO) in BRB80 buffer (80 mM Pipes, pH 6.9, 1 mM EGTA, 1 mM MgCl$_2$) with 4 mM MgCl$_2$, 1 mM MgGTP, and 5% DMSO at 37 °C for 30 min. Microtubules were then diluted in BRB80 buffer supplemented with10 μM taxol.

Preparation of multi-layer film coated CaCO$_3$ microspheres:

Preparation of CaCO$_3$ microspheres was prepared as follow: 1 ml of 0.33 M Na$_2$CO$_3$ was first injected in a round flask. Then same volume of 0.33 M CaCl$_2$ solution was added quickly to the above solution with intense agitation on a magnetic stirrer
(1700 rm min\(^{-1}\)). After 25 s, this system was allowed to stand for 2 min. The products were washed with deionized water thoroughly for three times.

For the assembly process, CaCO\(_3\) microspheres were firstly dispersed into 2 mg mL\(^{-1}\) PAH in 0.3 M NaCl solution for 10 min, followed by three times centrifugation and washing with 0.3 M NaCl solution. Then the microspheres were dispersed into 2 mg mL\(^{-1}\) PSS in 0.3 M NaCl solution for 10 min, followed by three times centrifugation and washing with 0.3 M NaCl. After the assembly of the desired number of PAH/PSS layers, the coated microspheres were incubated in 0.01 M ATP solution and shaken at room temperature overnight for the adsorption of ATP.

**Characterization**

The size and surface structure of multi-layer film coated CaCO\(_3\) microspheres were characterized by scanning electron microscope (SEM, Hitachi S-4800). CLSM micrographs were taken with an Olympus FV1000MPE confocal system equipped with 60× oil-immersion objective and a numerical aperture of 1.4.

**Measurement of ATP loading efficiency:**

ATP loading efficiency was calculated through the standard curve method. Hydrochloric acid was added to 150 µl of ATP loaded microspheres dispersion to totally decompose the microspheres. The obtained solution was then diluted to a volume of 900 µl and tested with UV spectrophotometer to record the UV absorbance at 260 nm. ATP concentration of the obtained solution was calculated according to the standard curve of ATP concentration.

**Measurement of ATP release:**
Ultraviolet-Visible spectroscopy: The catalase-glucose oxidase-glucose mixture triggered ATP release from the multi-layer film coated microspheres was analyzed by the UV-VIS measurement. The ATP-loaded CaCO₃ microspheres were first dispersed in BRB80 buffer with 100 mM glucose, 0.2 mg mL⁻¹ glucose oxidase, 0.08 mg mL⁻¹ catalase. Then the UV-VIS spectroscopy of the microspheres supernatant was recorded every 30 min. As comparison, same measurement was taken with microspheres dispersed in pure BRB80 buffer, where there was no CAT-GOD-G mixture.

As ATP shows remarkable UV absorbance peak at 260 nm, we took the absorbance intensity at 260 nm as a standard to quantitatively analyze the ATP release from multi-layer film coated CaCO₃ microsphere in CAT-GOD-G mixture. ATP-loaded CaCO₃ microspheres were dispersed in BRB80 buffer with 100 mM glucose, 0.2 mg mL⁻¹ glucose oxidase, 0.08 mg mL⁻¹ catalase, and after centrifugation, the absorbance intensity of the supernatant at 260 nm was recorded. This centrifugation and UV measurement was repeated every 30 min. The concentrations of ATP at different times were calculated according to the ATP concentration standard curve.

*Luminescence intensity measurement:*

ATP release was also measured via an ENLITEN®ATP Assay System (Promega, FF2000) in a BPCL ultraweak chemiluminescence analyzer connected to a chart recorder (BPCL-1-TIC, Institute of Biophysics, Chinese Academy of Sciences, Beijing). As the luminescence intensity of luciferin is proportional to the amount of ATP, the increase of luminescence intensity can also indicate release of free ATP in solution. The determinations were performed at room temperature in dark. To analyze the free
ATP concentration, the ATP-loaded CaCO₃ microspheres were first dispersed into BRB80 buffer supplement with 100 mM glucose, 0.2 mg mL⁻¹ glucose oxidase, 0.08 mg mL⁻¹ catalase. Then every 30 min, 5 μL of the microspheres supernatant was added into a luminometer cuvette and mixed with 50 μL of luciferin-luciferase reagent (Promega, FF2000), immediately followed by registering the luminescence intensity on a recorder of BPCL ultraweak chemiluminescence analyzer.

*Fluorescence experiments:*

Fluorescence measurement was taken to verify the pH changes in the catalase-glucose oxidase-glucose mixture. 1 ml 0.2 mg mL⁻¹ glucose oxidase, 1 ml 0.08 mg mL⁻¹ catalase and 20 μl 20 mM pyranine were first mixed in the cuvette, then 1 ml 100 mM glucose was quickly added to the above solution. The fluorescence intensity was observed immediately after solution was mixed thoroughly. The fluorescence intensity was recorded every 30 min.

*Preparation of the motility solution:*

The solution for the motility assay was prepared as follows: the CaCO₃ microspheres dispersion was added with 5 μL rhodamine labeled microtubules and the oxygen scavenger (100 mM glucose, 0.2 mg mL⁻¹ glucose oxidase, 0.08 mg mL⁻¹ catalase, 0.5 μL Taxol and 0.5 μL 0.5% β-mercaptoethanol, respectively.). The total volume of the mixture was kept to a total volume of 100 μL with BRB80 buffer.

*Construction of flow chambers and motility assays:*

Motility experiment was performed in the flow chambers with a volume of about 10 μL. The chambers were assembled with a glass slide as the bottom surface, a
coverslip to cover the surface, and two strips of double-sided tape as spacer. The surfaces of the chambers were first coated with 0.5 mg mL⁻¹ casein solution (in BRB80 buffer) on ice for 5 min, and then with 10 μg mL⁻¹ kinesin solution (in BRB80 buffer) for another 5 min, finally the chambers were perfused with the motility solution. They were sealed with silicone and observed under the confocal laser scanning microscope (Olympus FV500) with a 60xoil-immersion objective.

**Supplementary Results:**

*Detection of pH changes:*

To verify CAT-GOD-G mixture could lead to the decomposition of CaCO₃ microspheres, we first test the pH changes with time in the CAT-GOD-G mixture with pyranine, the commonly used pH probe. With the pH decrease, the fluorescence intensity of pyranine at 406 nm will increase while the intensity at 460 nm will decrease. Fig. S1 shows that fluorescence intensity trends of pyranine in the CAT-GOD-G mixture fits well of the theory, which indicates the pH decease in the mixture.

*Characterization of the decomposition of CaCO₃ microspheres with CAT-GOD-G mixture:*

To further confirm the decomposition of CaCO₃ in the presence of CAT-GOD-G mixture, we studied the morphology of assembled CaCO₃ microspheres. Fig. S2 gives the morphology of the microspheres in CAT-GOD-G mixture at different times. By diffusing the microspheres in the mixture for 30 min, we can observe the slight
decomposition of these microspheres, which vaguely showed wrinkles of the outside assembly multilayer films. An hour later, the wrinkles became obvious and after 90 min, more obvious wrinkles could be observed. With the time increase, we could even find the complete decomposition of CaCO₃ microspheres (120 min), resulting in the hollow capsule. All the above results demonstrate that the CAT-GOD-G mixture can lead to the decomposition of CaCO₃ microspheres.

**Supplementary Figures:**

**Fig. S1** Characterization of the pH changes caused by CAT-GOD-G mixture with time.

**Fig. S2** Morphology changes of the assembled CaCO₃ microtubule in the presence of CAT-GOD-G mixture (The scale bars are 500 nm.)
Fig. S3 Standard curve of ATP concentration.

Supplementary Movies:

Mov. S1 The average velocity of MT gliding with CAT-GOD-G mixture. (5 min, 9±2 nm s$^{-1}$)

Mov. S2 The average velocity of MT gliding with CAT-GOD-G mixture. (30 min, 54±1 nm s$^{-1}$)

Mov. S3 The average velocity of MT gliding with CAT-GOD-G mixture. (60 min, 86±5 nm s$^{-1}$)

Mov. S4 The average velocity of MT gliding with CAT-GOD-G mixture. (120min, 120±10 nm s$^{-1}$)

Mov. S5 The average velocity of MT gliding without CAT-GOD-G mixture. (120 min)

References: