Electronic Supplementary Material (ESI) for Materials Advances.

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Catalase-driven protein microtube motors with different exterior surfaces as ultrasmall biotools

Mizuki Umebara, Natsuho Sugai, Kohei Murayama, Tomonao Sugawara, Yushi Akashi, Yoshitsugu Morita, Ryo Kato and Teruyuki Komatsu*

Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, 1-13-27 Kasuga, Bunkyo-ku, Tokyo 112-8551, Japan

Corresponding author: Prof. Teruyuki Komatsu

Tel & Fax: +81-3-3817-1910, E-mail: komatsu@kc.chuo-u.ac.jp

Experimental section

F-*E. coli* survival tests in the HEPES solution containing H_2O_2 and Triton X-100

The F-*E. coli* suspension (OD_{600} = 0.05, 0.2 mL) was added to the HEPES solution (pH 6.8, 10 mM, + 1 mM $CaCl_2$, +1 mM $MnCl_2$, 0.8 mL) (working solution) and the resultant mixture ([F-*E. coli*] = 5.0×10^6 cells/mL) was incubated for 5, 15, 30, 60, 90, 120, 150, and 180 min. After centrifugation (4000 × *g*, 10 min), the supernatant was removed and the precipitated cells were redispersed in HEPES solution (1 mL). Then the fluorescent spectrum was measured (λ_{ex}, 395 nm). The fluorescent intensity (λ_{em}, 508 nm) of the F-*E. coli* sample exposed in the working solution for 15 min was 93% of that of the control sample (without exposure to the working solution). In contrast, the fluorescent intensity of the F-*E. coli* sample exposed in the working solution for 180 min was 48% of the control.

The F-*E. coli* suspension (OD_{600} = 4.00, 0.2 mL) was added to the working solution and mixed well ([F-*E. coli*] = 4.0×10^8 cells/mL). After 5, 15, and 30 min, the sample (50 μL) was pipetted out and dispersed into saline solution (50 mL). After centrifugation (1500 × *g*, 30 min, 4 ºC), the precipitated cells were dispersed in LB/ampicillin medium (5 mL) and the obtained mixture was incubated in a shaking incubator at 37 ºC. To observe the culturability, the OD_{600} value was monitored for 24 h. The OD_{600} of the F-*E. coli* sample exposed in the working solution for 15 min reached the same value of the control sample (without exposure to the working solution) after 24 h. In contrast, the OD_{600} of the sample exposed in the working solution for 30 min reached only 70% of the control. These results suggest that a large fraction of F-*E. coli* in the working solution were expected to remain viable and culturable within 15 min. Certainly, these survival tests have limitation. However, the Con A–LPS binding allows the tube to capture F-*E. coli* regardless of their bacterial viability or calturability.

Synthesis of αGD-covered Cat MTs (αGD/Cat MTs)

The Avi MTs (ca. 8.23×10^6 tubes, one-sixth of the powder prepared using one PC membrane) were dispersed in deionized water (1.7 mL) using an ultrasonic cleaner for 1 min. To this dispersion, the 20× PBS solution (pH 7.4, 0.1 mL) and 9% NaCl solution (195 μL) was added, and the mixture was incubated for 30 min at 25°C. Then the PB solution (pH 7.0, 10 mM, 4.74 μL) of bCat (15.9 μM) was

injected into the dispersion, and the resultant mixture (ca. 4.12×10^6 tubes/mL, [bCat] = 37.7 nM, [bCat]/[Avi] = 2.0 (mol/mol), PBS +150 mM NaCl, 2 mL) was incubated for 3 h at 25°C under the darkness. The Cat MTs were collected at the bottom using a Nd-magnet and the supernatant was removed. The precipitated tubes were resuspended in PB solution (2 mL) and collected again by a magnet. After removing the supernatant, PB solution was added to adjust the volume of 0.35 mL (ca. 2.35×10^7 tubes/mL). Subsequently, the PB solution of αGD (1 mg/mL, 0.15 mL) was added and the obtained mixture was incubated for 30 min at 25°C. Then the MTs were collected by a magnet and the supernatant was discarded, followed by adding PB solution (0.5 mL). By repeating this washing cycle two times, we obtained the PB solution (0.5 mL) of αGD/PLA/HSA/MNP(PLA/HSA)$_5$PLA/PLG/Avi/bCat MTs (αGD/Cat MTs). Using the same procedure, αGD/PLA/HSA/MNP(PLA/HSA)$_5$PLA/PLG MTs (αGD/PLG MTs) were also prepared.

Coverage rate estimation with αGD

The coverage rate of the outer surface of Cat MTs with αGD was estimated by the same procedure of CyConA/Cat MTs using a fluorescent FαGD. To the PB solution (pH 7.0, 10 mM, 0.35 mL) of Cat MTs (ca. 2.35×10^7 tubes/mL) prepared as described above, the PB solution of FαGD (0.04 mg/mL, 0.15 mL) was added and the mixture was incubated for 30 min at 25°C. The FαGD/Cat MTs were then collected by a Nd-magnet, and the fluorescent spectrum of the supernatant (0.1 mL) was measured (λ_{ex}, 495 nm / λ_{em}, 525 nm) to ascertain the concentration of the unbound FαGD. An identically treated control FαGD sample without MT was prepared; its fluorescence intensity was regarded as a 100% FαGD concentration.

Maximum amount of proteins absorbed on the exterior surface of Cat MT

Maximum amount of protein (ConA, αGD, HRP) (N mol) that can be absorbed on the exterior surface of Cat MT was calculated using (eqn (S1)).

$$N = \left\{\pi\left({}^{D}/_{2} + T\right)^2 - \pi\left({}^{D}/_{2}\right)^2\right\} \times L \times d \,/ Mw \qquad \text{(S1)}$$

D: O.D. of Cat MT in swelled state in water.

T: Thicknesses (diameter) of protein absorbed on the exterior surface of Cat MT in swelled state in water.

L: T.L. of Cat MT.

d: Density of protein absorbed on the exterior surface of Cat MT.

M_w: Molecular weight of protein absorbed on the exterior surface of Cat MT.

Synthesis of HRP-covered Cat MTs (HRP/Cat MTs)

The PB dispersion (pH 7.0, 10 mM, 2.0 mL) of Cat MTs (ca. 4.12×10^6 tubes/mL) was prepared as described above. Using a Nd-magnet, the MTs were collected at the bottom and the supernatant was removed. Subsequently, the precipitated tubules were resuspended in PB solution of HRP (0.2 μM, 0.5 mL) and the mixture was incubated for 30 min at 25°C. The MTs were attracted by a magnet and the supernatant was discarded, followed by adding PB solution (0.5 mL). By repeating this washing two times, we obtained the PB solution (0.5 mL) of HRP/PLA/HSA/MNP(PLA/HSA)$_5$ PLA/PLG/Avi/bCat MTs (HRP/Cat MTs). Using the same procedure, HRP/PLA/HSA/MNP(PLA/HSA)$_5$PLA/PLG/Avi/ HSA MTs (HRP/HSA MTs) were prepared from HSA MTs.

Coverage rate estimation with HRP

The coverage rate of the outer surface of Cat MTs with HRP was estimated by the same procedure of FαGD/Cat MTs using a fluorescent CyHRP. An identically treated control CyHRP sample without MTs was prepared; its fluorescence intensity was regarded as a 100% CyHRP concentration.

Synthesis of AuNP-coated Cat MTs (AuNP/Cat MTs)

The another precursor MTs [(PLA/HSA)$_7$PLA/PLG/Avi MTs] without MNP layer were synthesized according to the same procedure described in materials and methods section using a PC membrane (1.2

μm pore-diameter, 4.94×10^7 pores/piece; Millipore Corp.) (Fig. S4). The MTs (ca. 8.23×10^6 tubes, one-sixth of the powder prepared using one PC membrane) were dispersed in deionized water (1.7 mL) using an ultrasonic cleaner for 1 min. This dispersion was divided into two. To each dispersion, the 20× PBS solution (pH 7.4, 50 μL) and 9% NaCl solution (97.5 μL) was added, and the mixture was incubated for 15 min at 25°C. Then, the PB solution (pH 7.0, 10 mM, 2.2 μL) of bCat (15.9 μM) was injected to the dispersion, and the resultant mixture (ca. 4.12×10^6 tubes/mL, [bCat] = 35 nM, [bCat]/[Avi] = 1.9 (mol/mol), PBS +150 mM NaCl) was incubated for 3 h at 25°C under the darkness. After centrifugation (1000 × *g*, 10 min), the supernatant including unbound bCat was discarded. The precipitated $(PLA/HSA)_7$PLA/PLG/Avi/bCat MTs (Cat2 MTs) were redispersed in deionized water and centrifuged again (1000 × *g*, 10 min). After removing the supernatant, the precipitated tubes in 50 μL solution were suspended in aqueous dispersion of AuNP (0.25 mg/mL, 0.5 mL) and the mixture (0.55 mL) was incubated for 30 min at 25°C. Then the dispersion was centrifuged (500 × *g*, 1 min) to remove the supernatant and the precipitated MTs were dispersed in deionized water (0.5 mL). The dispersion was centrifuged again (500 × *g*, 1 min) and the supernatant was removed. Next, the precipitate MTs were suspended in PB solution (pH 7.0, 10 mM) and centrifuged (500 × *g*, 1 min) to remove the supernatant. After repeating this washing with PB solution two times, the volume was adjusted to 2.0 mL, yielding AuNP/$(PLA/HSA)_7$PLA/PLG/Avi/bCat MTs (AuNP/Cat MTs).

Coverage rate estimation with AuNP

The initial supernatant (0.3 mL) removed from the mixture solution of Cat2 MTs and AuNPs after the centrifugation was diluted with 1.2 mL water (5-fold dilution). The UV-vis absorption spectrum of this solution was measured to assay the concentration of the unbound free AuNPs. An identically treated control AuNP sample without MTs was also prepared; its absorption intensity at 530 nm was regarded as a 100% AuNP concentration.

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), and confocal laser scanning microscopy (CLSM)

SEM, TEM, and CLSM observations of the MTs were performed as described in our previous paper.[26] The SEM measurements were conducted using a field-emission scanning electron microscope (S-4300; Hitachi High-Technologies Corp.) with an accelerating voltage of 10 kV. The TEM measurements were performed using a transmission electron microscope (HT-7700; Hitachi High-Technologies Corp.) with an accelerating voltage of 100 kV. The CLSM measurements were carried out using a laser scanning microscope (LSM 510; Carl Zeiss Inc.). Fluorescein labeled materials were imaged using Ar^+ laser (λ_{ex}, 488 nm / LP505 filter) and Cy5.5 labeled materials were imaged using He-Ne laser (λ_{ex}, 633 nm / LP650 filter).

Digestion of MTs

The PB solution (pH 7.0, 10 mM, 50 μL) of ConA/Cat MTs, αGD/Cat MTs, HRP/Cat MTs, and AuNP/Cat MTs were mixed, respectively, with Tris-HCl buffer solution (pH 7.5, 50 mM, 50 μL) of Pronase (Roche Diagnostics GmbH) (5 mg/mL). The 6 μL of the mixture was placed onto the slide glass and was sealed by a cover glass with colorless resin. The time-course of the morphology change at 37 ºC was observed using a research inverted microscope. After 2 h, the mixture was sonicated briefly and was observed using microscope.

Results

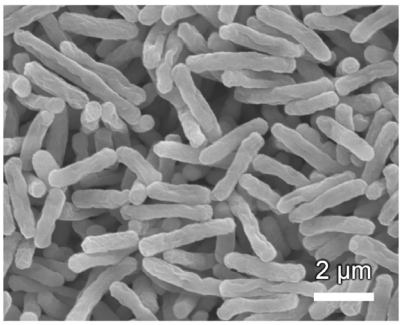

Fig. S1 SEM image of F-*E. coli*.

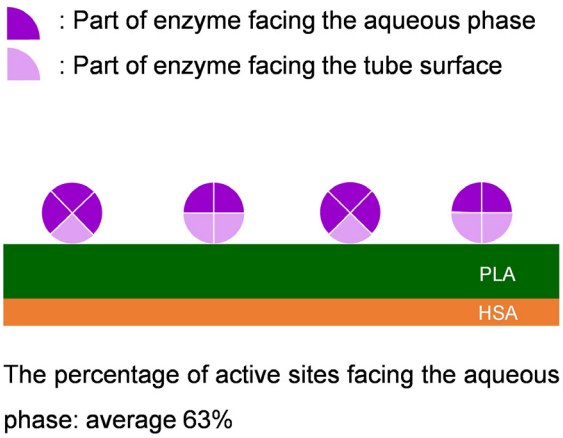

Fig. S2 The proposed geometries of αGD absorbed statistically on the exterior surface of Cat MT.

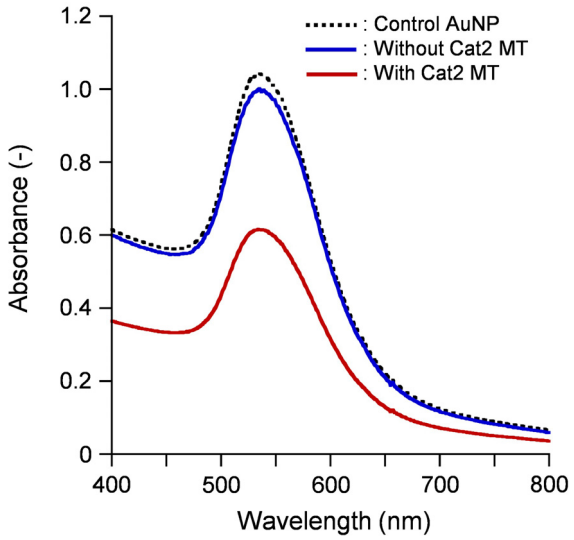

Fig. S3 Visible absorption spectra of the AuNP dispersion (PB, pH 7.0) after the treatment with Cat2 MTs, with subsequent centrifugation.

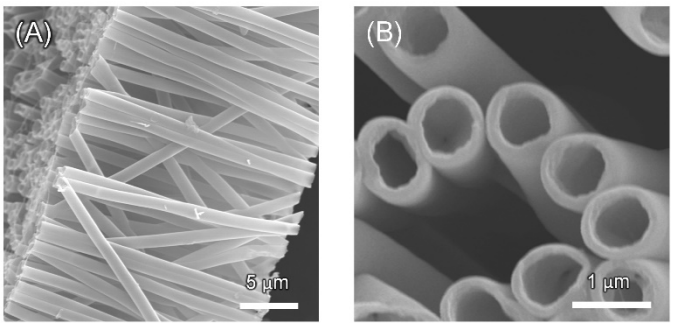

Fig. S4 SEM images of $(PLA/HSA)_7PLA/PLG/Avi$ MTs.

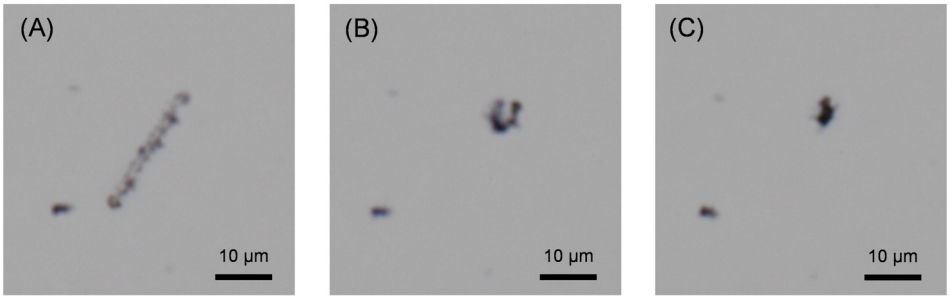

Fig. S5 Morphology change of AuNP/Cat MT in Pronase solution (pH 7.5), (A) at 0 min, (B) after 15 min, and (C) after 2 h at 37 °C. The images were observed by optical microscopy.

Video S1 Turning motion of self-propelled ConA/Cat MT by jetting O_2 bubbles in HEPES solution (pH 6.8, 10 mM, 2 wt% H_2O_2, 0.1 wt% Triton X-100) at 25 °C.

Video S2 Turning motion of self-propelled αGD/Cat MT by jetting O_2 bubbles in PB solution (pH 7.0, 10 mM, 2 wt% H_2O_2, 0.1 wt% Triton X-100) at 25 °C.

Video S3 Turning motion of self-propelled HRP/Cat MT by jetting O_2 bubbles in PB solution (pH 7.0, 10 mM, 2 wt% H_2O_2, 0.1 wt% Triton X-100) at 25 °C.

Video S4 Turning motion of self-propelled AuNP/Cat MT by jetting O_2 bubbles in PB solution (pH 7.0, 10 mM, 2 wt% H_2O_2, 0.1 wt% Triton X-100); (A) without light irradiation, (B) under the light irradiation (139 mW/cm^2) at room temperature.