=Electronic supplementary information=

Swimming protein microtube motors capture virus-shaped fluorescent nanoparticles

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Experimental section

Materials and apparatus

Fetuin (from fetal bovine serum), poly(L-arginine) hydrochloride (PLA; Mw, >70,000), and poly(L-glutamic acid) sodium salt (PLG; Mw, 50,000–100,000) were purchased from Merck KGaA. Catalase from bovine liver (Cat), polyoxyethylene(10) octylphenyl ether (Triton X-100), and *N*-hydroxy succinimide (NHS) were purchased from Fujifilm Wako Pure Chemical Corp. Human serum albumin (HSA) was purchased from the Japan Blood Products Organization. Avidin (Avi) was purchased from Calzyme Laboratories, Inc. The magnetite (Fe_3O_4) nanoparticle (MNP) (EMG607, ca. 10 nm diameter covered with cationic surfactant) was purchased from Ferrotec Corp. Biotinylated catalase (bCat) and biotinylated HSA (bHSA) were prepared according to a previously reported procedure.[16,24] Fluorescein-labeled Cat (FCat) was prepared by general procedure using fluorescein isothiocyanate (FITC; Merck KGaA). Cyanine dye-labeled fetuin (CyFet) was prepared by general procedure using cyanine5.5 NHS ester (Cy5.5; Lumiprobe Corp.); Cy5.5/fetuin was 0.5 mol/mol. Influenza A H1N1 (A/Puerto Rico/8/34) hemagglutinin having a histidine(His)-tag (HA) and hemagglutinin ELISA Kit (KIT11684) were purchased from Sino Biological, Inc. Anti-hemagglutinin(epitope)-tag pAb Alexa Fluor® 488 (polyclonal, Rabbit IgG) (FIgG) was purchased from Medical & Biological Laboratories Co., Ltd. (5S)-*N*-(5-Amino-1-carboxypentyl)iminodiacetic acid (NTA) was purchased from Combi-Blocks Inc. Deionized water (18.2 M·cm) was prepared using a water purification system (Milli-Q IQ7003 system; Merck KGaA). UV-Vis absorption spectra were recorded using a UV/Vis spectrophotometer (8453; Agilent Technologies Inc. or V-650; Jasco Corp.). Fluorescence emission spectra were recorded using a spectrofluorometer (FP-8300; Jasco Corp.).

Preparation of Fet-covered Cat MTs (Fet/Cat MTs)

The precursor PLA/HSA/MNP(PLA/HSA)$_5$PLA/PLG/Avi microtubes (Avi MTs) were prepared according to a previously reported procedure using a track-etched polycarbonate (PC) membrane (Isopore membrane, 25 mm diameter, 1.2 μm pore-diameter, 4.94×10^7 pores/piece; Millipore Corp.).[16,24] The Avi MTs were obtained as lyophilized pale-yellow powder.

The Avi MTs (ca. 8.23×10^6 tubes, one-sixth of the powder prepared using one PC membrane) were dispersed in deionized water (3.41 mL) using an ultrasonic cleaner for 1 min. This aqueous dispersion was divided into two. After the 20× phosphate-buffered saline (PBS) solution (pH 7.4, 0.1 mL) and 9% NaCl solution (195 μL) were added to each dispersion, the mixture was incubated for 30 min at 25°C. Then, the PB solution (pH 7.0, 10 mM, 1.3 μL) of bCat (26.2 μM) was added into the dispersion. The resultant mixture (ca. 2.06×10^6 tubes/mL, [bCat] = 17 nM, [bCat]/[Avi] = 1.8 (mol/mol), PBS +150 mM NaCl, 2 mL) was incubated for 3 hr at 25°C under the dark. The dispersion was centrifuged (500 × *g*, 3 min) to remove the supernatant including free bCat. The precipitated MTs, PLA/HSA/MNP(PLA/HSA)$_5$PLA/PLG/Avi/bCat MTs (Cat MTs) were suspended in PB solution (pH 7.0, 10 mM, 2 mL) and centrifuged again (500 × *g*, 3 min). After discarding the supernatant, PB solution was poured to adjust the volume of 0.35 mL. Subsequently, the PB solution of Fet (1 mg/mL, 0.15 mL) was added and the mixture was incubated for 30 min at 25°C. The dispersion was centrifuged (500 × *g*, 3 min) and the supernatant was removed. The precipitate was redispersed in PB solution (2 mL). By repeating this washing two-times, we obtained the PB solution (2 mL) of Fet/PLA/HSA/MNP(PLA/HSA)$_5$PLA/PLG/Avi/bCat MTs (Fet/Cat MTs). Using the same procedure, Fet/PLA/HSA/MNP(PLA/HSA)$_5$PLA/PLG MTs (Fet/PLG MTs) and HSA/PLA/HSA/MNP (PLA/HSA)$_5$PLA/PLG/Avi/bCat MTs (HSA/Cat MTs) were prepared.

Amount of Fet adhered on Cat MT

The amount of adhered Fet on the exterior surface of Cat MT (*N*) was determined using a fluorescent CyFet. The Cat MTs were prepared in the manner described above (ca. 2.06×10^6 tubes/mL, [bCat] = 17 nM, [bCat]/[Avi] = 1.8 (mol/mol), PBS +150 mM NaCl, 2 mL). The dispersion was centrifuged (500 × *g*, 3 min) to remove the supernatant including unbound bCat. The precipitated Cat MTs were suspended in PB solution (pH 7.0, 10 mM, 2 mL) and were centrifuged again (500 × *g*, 3 min). After removing the supernatant, PB solution was poured to adjust the volume of 0.9 mL. Subsequently, the PB solution of CyFet (0.4 μM, 0.1 mL) was added and the mixture was incubated for 30 min at 25°C. The dispersion of the CyFet/Cat MTs was centrifuged (4000 × *g*, 10 min). Then the fluorescence spectrum of the supernatant (0.8 mL) was measured (λ_{ex}, 684 nm / λ_{em}, 710 nm) to ascertain the

concentration of the unbound free CyFet. An identically treated control CyFet sample without MT was prepared; its fluorescence intensity was regarded as a 100% CyFet concentration. From these data, the amount of adhered Fet on Cat MT (N) can be calculated.

Maximum mount of Fet absorbed on Cat MT

Maximum amount of Fet that can be absorbed on the exterior surface of Cat MT (N_{max}) was calculated using (Eq. S1).

$$N_{max} = \{ \pi(D/2 + T)^2 - \pi(D/2)^2 \} \times L \times d / M_w \quad \text{(S1)}$$

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

T: Thicknesses (diameter) of Fet in swelled state in water.

L: T.L. of Cat MT.

d: Density of Fet.

M_w: Molecular weight of Fet.

From the ratio of adhered Fet (N) and calculated maximum amount of Fet (N_{max}) on the Cat MT, the coverage rate by Fet can be estimated.

Optical microscopy observations of swimming MTs

Typically, the PB dispersion (pH 7.0, 10 mM, ca. 1.06×10^6–4.12×10^6 tubes per mL, $[H_2O_2]$ = 2 wt%, [Triton X-100] = 0.1 wt%) of swimming MTs were prepared in a 24-well glass bottom microplate (Iwaki EZVIEW Culture Plate LB; AGC Techno Glass., Co., Ltd). In the condition of $[H_2O_2] < 2$ wt% and [Triton X-100] < 0.1 wt%, the MTs did not swim smoothly. The moving behavior was observed using a research inverted microscope (IX73; Olympus Corp.) under bright field mode equipped with a digital high-speed camera (HAS-220; Detect Corp.) at 200 frames/s. Editing of the movies and determination of the tube velocity were performed using an image processing program (Image J; NIH).

The observations under fluorescence mode were conducted a research inverted microscope with a digital single-lens reflex camera (IOS KissX7i; Canon Inc.)

Capture of influenza A H1N1 hemagglutinin having His-tag (HA) by Fet/Cat MTs

The PB dispersion (pH 7.0, 10 mM, 2 mL) of Fet/Cat MTs (ca. 2.06×10^6 tubes/mL) was prepared as described above. Using a Nd-magnet, the MTs were collected at the bottom and the supernatant was removed. The obtained Fet/Cat MTs were redispersed into PB solution (0.9 mL) containing H_2O_2 (2.22 wt%) and Triton X-100 (0.11 wt%) to initiate self-propulsion of the tubes. Thereafter, the PB solution (0.1 mL) of HA (90 nM) was slowly added. The obtained mixture (ca. 4.12×10^6 tubes/mL, [HA] = 9 nM, [HA]/[Fet] = 3 (mol/mol), [H_2O_2] = 2 wt%, [Trirton X-100] = 0.1 wt%, 1 mL) was left for 15 min without vibration at 25ºC. By exposure to a magnetic field using a Nd-magnet, the HA bound Fet/Cat MTs (HA-Fet/Cat MTs) were collected at the bottom and the supernatant was transferred to other plastic tube. This treatment was repeated three-times to remove the MTs completely. Then ELISA measurements of the supernatant was conducted using hemagglutinin ELISA kit to determine the concentration of the unbound free HA. An identically treated control sample without MT was also prepared; its HA concentration was regarded as 100%. The same experiments were conducted using the Fet/PLG MTs and HSA/Cat MTs.

Binding of FIgG to the HA-Fet/Cat MTs

To the PB dispersion (pH 7.0, 10 mM, 0.5 mL) of the Fet/Cat MTs (ca. 1.65×10^6 tubes/mL), the PB solution (5.4 μL) of HA (8.47 μM) was injected and the mixture was incubated with rotation for 30 min at 25ºC. Subsequently, the PB solution (3.1 μL) of FIgG was added and the mixture was incubated for 10 min. After centrifugation (500 × *g*, 3 min), the supernatant was discarded and the MTs were redispersed into the PB solution (1 mL), yielding FIgG bound HA-Fet/Cat MTs (FIgG-HA-Fet/Cat MTs).

The obtained PB dispersion (0.25 mL) of FIgG-HA-Fet/Cat MTs and PB dispersion (pH 7.0, 10 mM, 0.25 mL) of H_2O_2 and Triton X-100 ([H_2O_2] = 4 wt%, [Triton X-100] = 0.2 wt%) were mixed in a 24-well glass bottom microplate. The moving behavior was observed using a research inverted

microscope under fluorescence mode and was recorded using a digital single-lens reflex camera (IOS KissX7i; Canon Inc.).

Synthesis of hemagglutinin-immobilized fluorescent nanoparticles (HA-FNP)

The fluorescent polystyrene nanoparticles with carboxyl groups on the surface (Fluoresbrite YG carboxylate microspheres, diameter: 100 nm, 27 mg/mL, 4.55×10^{13} particles/mL, λ_{ex}: 441 nm / λ_{em}: 486 nm) (FNP) were purchased from Polysciences, Inc. All experiments shown below were conducted in the dark as much as possible. The PB solutions of EDC (25.9 mM, 24.0 μL) and NHS (45.7 mM, 13.6 μL) were added to the PB dispersion (pH 7.0, 10 mM, 4.95 mL) of FNP (2.53×10^{12} particles/mL), and the obtained mixture was stirred for 1.5 h at 25 ºC. Subsequently, the PB solution of NTA (20.7 mM, 10 μL) was injected, followed by stirring for 15 h at 25 ºC. The resultant dispersion was dialyzed for 16 h at 4 ºC against 100-fold volumes of PB, yielding NTA-FNP (2.50×10^{12} particles/mL). The PB solution of $NiCl_2$ (9.6 mM, 3.5 μL) was then added to the NTA-FNP dispersion (5 mL, 2.02×10^{12} particles/mL), and the resultant was mixed for 1 h at 25 ºC with rotation. Finally, the PB solution of HA (1.81 μM, 24.4 μL) was added to the Ni-NTA-FNP dispersion (976 μL, 6.91×10^{10} particles/mL), followed by incubation for 2 h at 25 ºC. The particle dispersion was dialyzed for 20 h at 4 ºC against 300-fold volumes of PB, providing HA-FNP (6.74×10^{10} particles/mL).

Capture of HA-FNP by Fet/Cat MTs

The PB dispersion (pH 7.0, 10 mM, 0.6 mL) of Fet/Cat MTs (ca. 1.15×10^7 tubes/mL) was prepared as described above. To this dispersion, the PB solution (0.2 mL) containing H_2O_2 (10 wt%) and Triton X-100 (0.5 wt%) was added to initiate self-propulsion of the tubes. Thereafter, the PB solution of HA-FNP (6.74×10^{10} particles/mL, 0.2 mL) was slowly injected. The obtained mixture (ca. 6.90×10^6 tubes/mL, [H_2O_2] = 2 wt%, [Trirton X-100] = 0.1 wt%, 1 mL) was left for 2 h without vibration at 25ºC. By exposure to a magnetic field using a Nd-magnet, the HA-FNP bound Fet/Cat MTs were collected at the bottom and the supernatant was transferred to other plastic tube. Fluorescence emission spectrum of this solution (λ_{ex}, 441 nm) was measured to ascertain the concentration of the unbound free HA-FNP. An identically treated control sample without MT was prepared; its fluorescence intensity was regarded

as a 100% HA-FNP concentration. The same experiments were performed using the Ni-NTA-FNP.

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.[16,17,24] 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).

Results

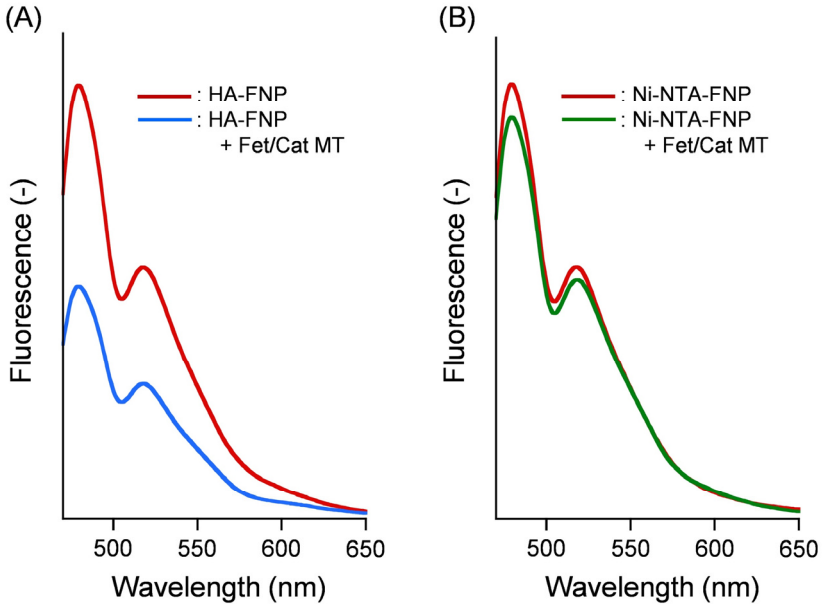

Fig. S1 Fluorescence spectra of the PB solution (pH 7.0, 1.0 wt% H_2O_2, 0.2 wt% Triton-X 100) of (A) HA-FNPs (λ_{ex}, 441 nm) after treatment with self-propelled Fet/CatNP MT motors and (B) Ni-NTA-FNPs (λ_{ex}, 441 nm) after treatment with self-propelled Fet/CatNP MT motors.

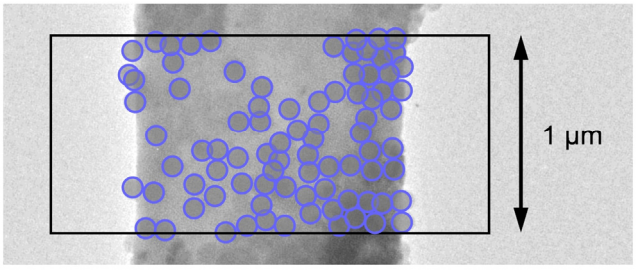

Fig. S2 TEM image of HA-FNP bound Fet/CatNP MT.

Video S1 Turning motion of self-propelled Fet/Cat MT by spouting 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 S2 Turning motion of self-propelled FIgG bound HA-Fet/Cat MT by spouting 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 under fluorescence mode.

Video S3 Turning motion of self-propelled HA-FNP bound HA-Fet/Cat MT by spouting 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 under bright field mode and fluorescence mode.