

**Supplementary Information**

**Near Infrared Light-Responsive Upconversion Nanoparticle Micromotor Propelled by Oxygen  
Bubbles**

Hanbee Kim and Sang-Yup Lee\*

Department of Chemical and Biomolecular Engineering, Yonsei University

50 Yonsei-ro, Seodaemun-gu, Seoul, Korea 120-749

## EXPERIMENTAL SECTION

### *Chemicals*

Yttrium oxide ( $Y_2O_3$ , 99.99%), ytterbium oxide ( $Yb_2O_3$ , 99.9%), erbium oxide ( $Er_2O_3$ , 99.99%), sodium trifluoroacetate ( $CF_3COONa$ , 98%), trifluoroacetic acid (TFA, 99%), oleic acid (90%), and 1-octadecene (90%). *N*-hydroxysuccinimide (NHS, 98%), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC, 99%), cysteamine (95%), tetra(4-carboxyphenyl)porphyrin (TCPP, 75%), iron(III) chloride hexahydrate ( $FeCl_3 \cdot 6H_2O$ , 97%) *p*-benzoquinone (BQ, 99.5%), poly(vinyl alcohol) (PVA, 99+% hydrolyzed), glucose (ACS reagent) and glucose oxidase (GOx, lyophilized, 100,000-250,000 units/g) were purchased from Sigma-Aldrich. Solvents including ethanol (extra pure grade), hexane (extra pure grade), chloroform (extra pure grade), *N,N*-dimethylformamide (DMF, 99.5%), isopropyl alcohol (IPA), hydrogen peroxide ( $H_2O_2$ , 30%), hydrochloric acid (lab grade), and sodium hydroxide (lab grade) were purchased from Duksan Chemical. All reagents and solvents were used as received.

### *Synthesis of NaYF<sub>4</sub> UCNP*

$NaYF_4$  UCNPs were synthesized according to a previously described protocol.<sup>1</sup> In brief, for the preparation of  $Ln(CF_3COO)_3$  ( $Ln = Y, Yb, Er$ ),  $Ln_2O_3$  was dissolved in 10 mL of 50% aqueous TFA and the mixture was refluxed at 70 °C until the liquid became clear. The residual water and acid were evaporated, and the  $Ln(CF_3COO)_3$  was obtained by vacuum drying at 70 °C. For the solvothermal synthesis, 15 mL of oleic acid and 15 mL of 1-octadecene were mixed in a three-neck flask. The mixture was heated to 120 °C to degas oxygen and water and subsequently heated to the thermolysis reaction temperature (310 °C) under a nitrogen environment. The precursor solution of  $NaYF_4$  was prepared by dispersing  $CF_3COONa$  (0.5 mmol),  $Y(CF_3COO)_3$  (0.39 mmol),  $Yb(CF_3COO)_3$  (0.1 mmol), and  $Er(CF_3COO)_3$  (0.01 mmol) in 10 mL of oleic acid and 10 mL of 1-octadecene mixture. After 10 min of vacuum de-gassing, the precursor solution was added dropwise to the flask at a volumetric rate of 1 mL/min, and the reaction was maintained at 310 °C for 1 h under nitrogen flow. Then, the mixture was cooled to room temperature and the UCNPs were precipitated by adding ethanol. The resulting white powders of UCNPs were washed and centrifuged with ethanol several times and finally dispersed in 10 mL chloroform for further use.

### *Modification of UCNP by the conjugation of TCPPFe*

Prior to the surface modification of UCNPs, the photosensitizer/catalyst of  $Fe^{3+}$ -coordinated TCPP (TCPPFe) was prepared. To obtain TCPPFe, a mixture of TCPP (9 mg) and  $FeCl_3 \cdot 6H_2O$  (10 mg) dissolved in 20 mL of DMF was refluxed at 150 °C for 2 h in a reaction flask.<sup>2</sup> Then, TCPPFe was precipitated by adding 100 mL of deionized water to the cooled reaction product. The TCPPFe precipitates were filtered, washed with deionized water, and then dried under vacuum at 65 °C.

For the conjugation of the photosensitizer, the surfaces of the synthesized UCNPs were modified with cysteamine via ligand exchange method to introduce amine groups. The ligand exchange was conducted according to a previously described protocol in literature.<sup>3</sup> A 4 mL dispersion of UCNPs (50 mg) in hexane and 6 mL of cysteamine (60 mg/mL) solution in ethanol were mixed for 48 h. The cysteamine-capped UCNPs (denoted as UCCs) were collected by centrifugation, washed several times with ethanol, and then dried in vacuum. The surface-exposed amine groups of UCCs were conjugated with TCPPFe via the NHS/EDC coupling reaction. In detail, EDC (4 mg) and NHS (6 mg) were added to a 4 mL dispersion of UCCs (9 mg) to activate the amine groups. To the dispersion, 20 mL of TCPPFe solution in DMF (0.55 mg/mL) was added to induce the coupling reaction. After magnetic stirring at room temperature for 36 h, the resulting product was collected by centrifugation, washed five times with ethanol, and then dried in vacuum.

#### *Detection of ROS generation*

Generation of reactive oxygen species was determined using methylene blue (MB) as an indicator. TUNP and MB solutions were mixed with and without H<sub>2</sub>O<sub>2</sub> solution. The decrease in MB absorbance at 664 nm was monitored using a UV–Vis spectrophotometer under NIR light irradiation for 30 min.

#### *Fabrication of TUNP motor*

The TCPPFe-decorated upconversion nanoparticles (TUNPs) were fabricated as a film by solution casting using a PVA matrix. PVA (1.5 g) was dissolved in 20 mL of deionized water at 80 °C under stirring, and the resulting solution was cooled to room temperature. A mixture of 1 mL PVA solution and 0.5 mL TUNP dispersion in water was drop-casted on a glass slide and dried at room temperature for 24 h to prepare a film, which was then cut into small pieces of 2 mm × 1 mm size for the light-driven motor experiments.

To use glucose, a natural fuel of many organisms, as a H<sub>2</sub>O<sub>2</sub> source, micromotor was modified to include glucose oxidase (GOx). The GOx-modified TUNP micromotor was prepared by adding 0.25 mL TUNP dispersion (1 mg/mL) and 0.25 mL GOx solution (4 mg/mL) to the 1 mL PVA matrix solution (75 mg/mL).

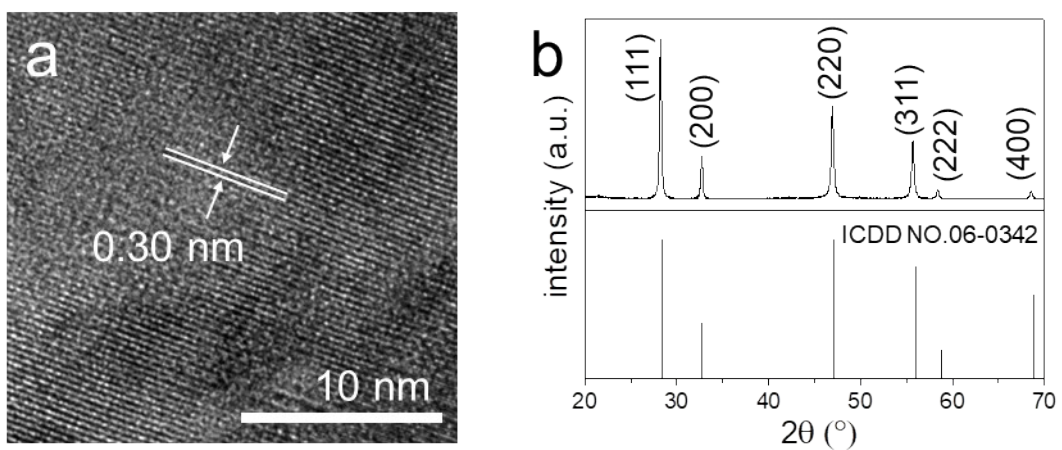
#### *Measurement of motor velocity and generated oxygen*

The velocity of the TUNP motor was determined by measuring the distance moved every 10 s. A digital microscope was used to record the movement of the motor in a Petri dish filled with H<sub>2</sub>O<sub>2</sub> solution. The velocity was calculated from the captured images taken every 10 s. The dissolved O<sub>2</sub> concentration in the solution was measured using a dissolved oxygen meter (HI-5421, Hanna

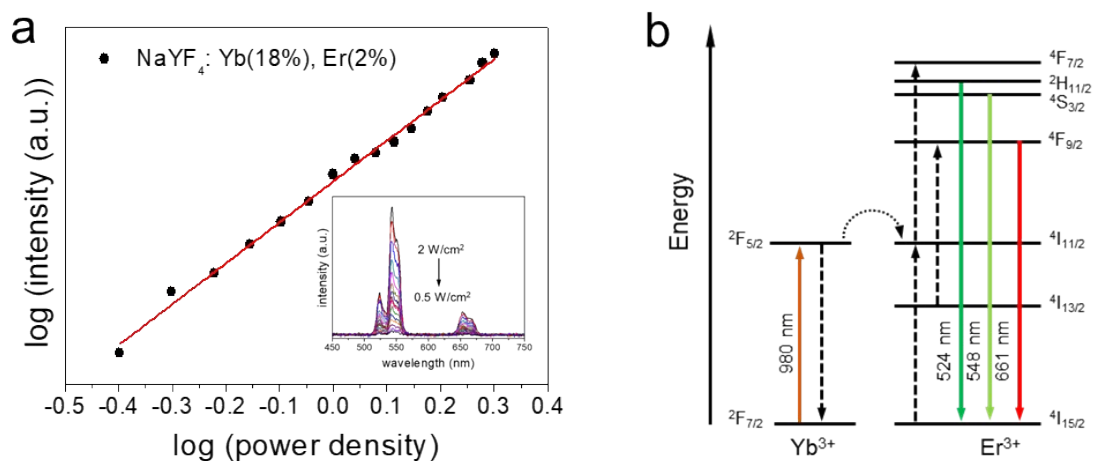
Instruments, Italy) both in the presence and absence of NIR light irradiation. The speed of the GOx-modified micromotor was measured at various glucose concentrations (0 – 100 mM).

#### *Characterization of the TUNP motor*

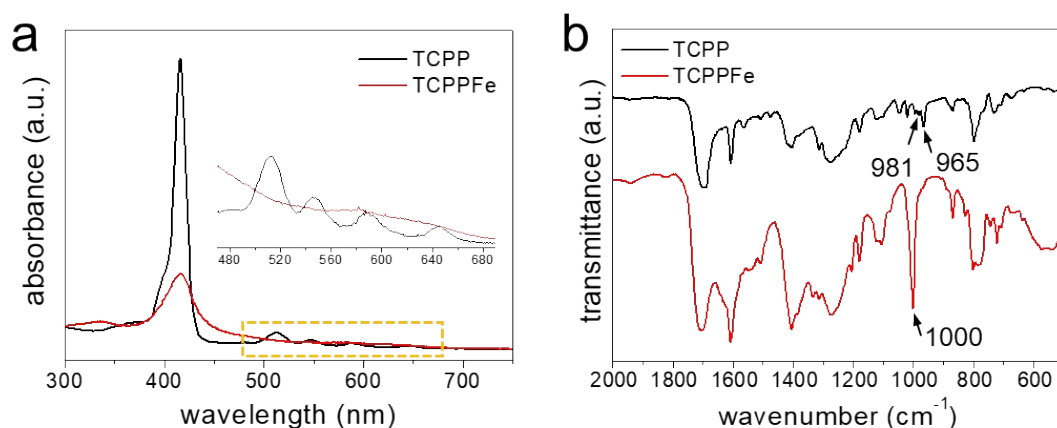
The morphology and chemical composition of the UCNPs were examined by transmission electron microscopy (TEM) and energy dispersive X-ray spectrometry (EDS) (JEM F200, JEOL Ltd., Japan, 200 kV). Scanning electron microscopy (SEM) images were obtained using an IT-500HR (JEOL Ltd., Japan). The crystalline structure of the UCNPs was analyzed from the powder X-ray diffraction (XRD) patterns measured by an X-ray diffractometer (Ultima IV, Rigaku, Japan, CuK $\alpha$  radiation;  $\lambda=0.15418$  nm). Dynamic light scattering and zeta potential measurements were performed by the electrophoretic light scattering method (ELS-2000ZS, Otsuka Electronics, Japan). FT-IR spectra were obtained using a Vertex 70 spectrometer (Bruker, Germany). X-ray photoelectron spectroscopy (XPS) data were collected using K-alpha (Thermo Fisher Scientific, US) equipped with Al K $\alpha$  radiation as the excitation source. UV-Vis absorbance spectra were recorded using a UV-Vis spectrophotometer (S-3100, Scinco, Korea). The luminescence spectra were obtained using a spectrofluorophotometer (RF-5301 PC, Shimadzu, Japan) equipped with an optical fiber coupled 980 nm diode laser (980 nm; output power 0–2 W/cm<sup>2</sup>). The optical images of the motor were recorded with an optical microscope (CK-2, Olympus, Japan). Motor motion was recorded using a digital microscope (OPT-500, Digibird, Korea). The pH was measured with a pH meter (Orion star A211, Thermo Fisher Scientific, US).



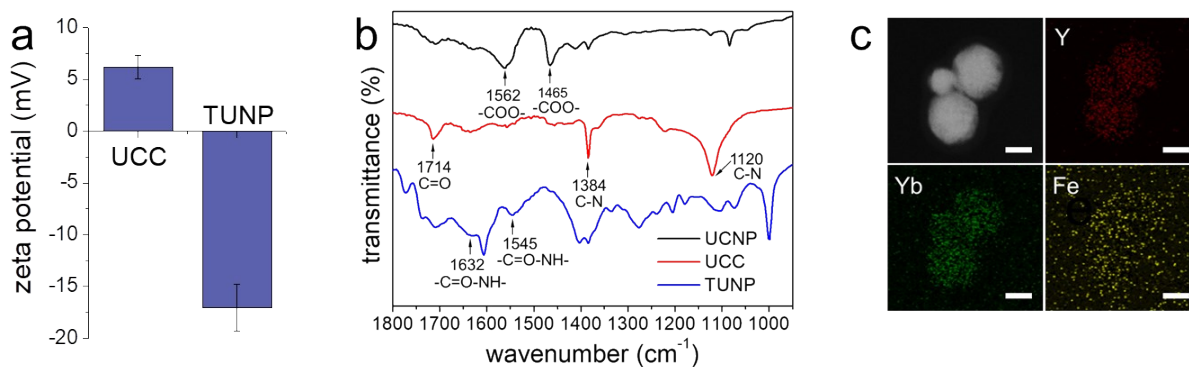
**Fig. S1** (a) High-resolution TEM image showing the correspondence of the lattice distances of 0.30 nm to the {111} plane of NaYF<sub>4</sub>. (b) XRD spectrum of UCNPs.



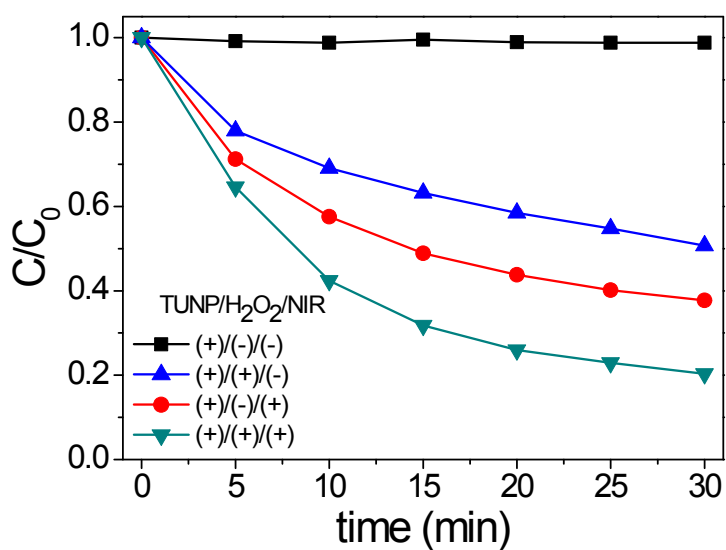
**Fig. S2** NIR light dependence of the upconversion luminescence of UCNPs. (a) Log–log plots of upconversion luminescence intensity at 543 nm versus excitation NIR power showing a slope of 2.06, indicating that this system is a two-photon process (Inset: Upconversion emission spectra of UCNPs under different power density from 0.5 to 2 W/cm<sup>2</sup>). (b) Schematic of upconversion excitation and visible emission of UCNPs.



**Fig. S3** Verification of Fe-coordination to tetra(4-carboxyphenyl)porphyrin (TCPP). (a) UV-Vis absorbance spectra of the TCPP and TCPPFe showing the reduced Q band from 4 to 1 band (inset), which is attributed to increased symmetry of the molecule when the  $\text{Fe}^{3+}$  ion coordinates with porphyrin nitrogen atoms. (b) FT-IR spectra of TCPP and TCPPFe. After coordination of  $\text{Fe}^{3+}$  ion, the Fe-N band appeared at  $1000\text{ cm}^{-1}$  and the N-H bending peaks located at  $965$  and  $981\text{ cm}^{-1}$  disappeared.

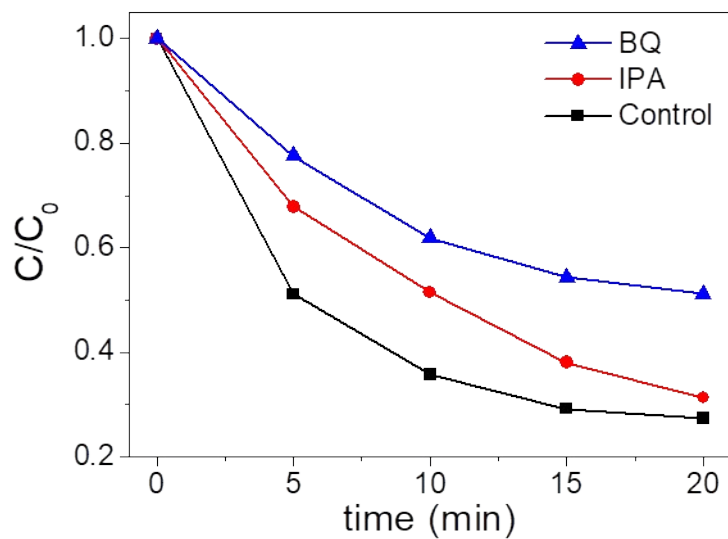


**Fig. S4** (a) Zeta potentials of UCCs and TUNPs. (b) FT-IR spectra of UCNPs, UCCs and TUNPs. (c) STEM and atomic mapping images of TUNPs (scale bars: 20 nm).

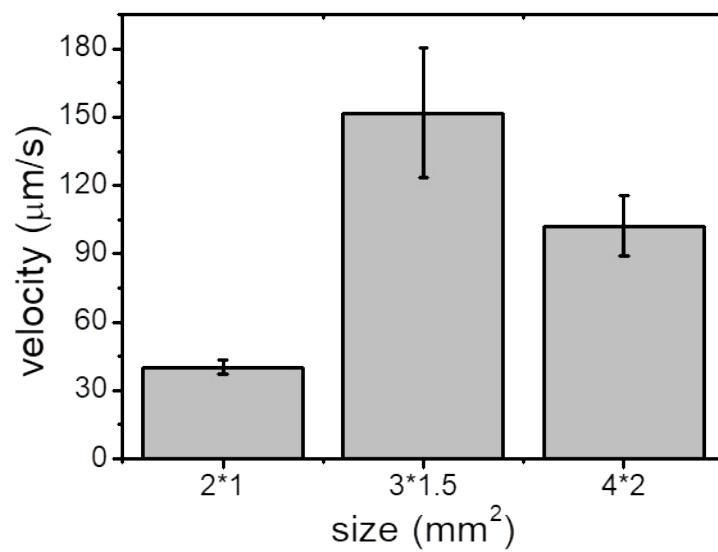


**Fig. S5** Plot of relative concentration change of MB as a function of time, showing the ROS generation at various conditions. MB concentration was monitored at  $\lambda_{\text{abs}} = 670$  nm. Experimental conditions: [MB]= 1.5 mg/L, [TUNP]= 0.1 g/L, [H<sub>2</sub>O<sub>2</sub>]= 0.5 mM. (+) and (-) signs of the legend represent presence and absence of the experimental elements, respectively.

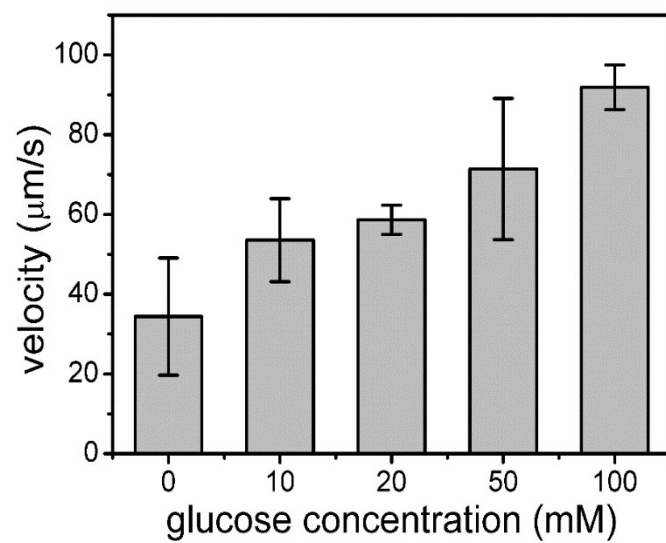




**Fig. S6** Effects of different scavengers on degradation of MB under NIR light irradiation. Experimental conditions: [MB] = 3.8 mg/L, [TUNP] = 0.1 g/L, [H<sub>2</sub>O<sub>2</sub>] = 0.38 mM, [Scavenger] = 7.7 mM. Control denotes the case where no scavenger was applied.



**Fig. S7** Velocity of TUNP micromotors in various sizes (TUNP concentration = 1 mg/ml; 5% H<sub>2</sub>O<sub>2</sub> solution, NIR light intensity: 0.4 W/cm<sup>2</sup>)



**Fig. S8** Speed of the GOx-modified micromotor at various glucose concentrations (NIR light intensity:  $0.4 \text{ W/cm}^2$ ).

## References

- 1 A. Punjabi, X. Wu, A. Tokatli-Apollon, M. El-Rifai, H. Lee, Y. Zhang, C. Wang, Z. Liu, E. M. Chan, C. Duan and G. Han, *ACS Nano*, 2014, **8**, 10621–10630.
- 2 G. Huang, T. M. Su, K. Zeng, Y. A. Guo, S. K. Zhao and S. J. Wei, *Appl Organomet Chem*, 2018, **32**, 1–11.
- 3 G. Ramírez-García, S. S. Panikar, T. López-Luke, V. Piazza, M. A. Honorato-Colin, T. Camacho-Villegas, R. Hernández-Gutiérrez and E. De La Rosa, *Nanoscale*, 2018, **10**, 10154–10165.