Electronic Supplementary Information (ESI)

In situ preparation of glyco-micromotors and its bacteria loading/guiding ability

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Experimental Section

Material

All the reagents are analytical grade. 2-Cyanoprop-2-yl-α-dithionaphthalate (CPDN) and 2-methacrylamido glucopyranose (MAG) were prepared as reported previously.¹,² 2-(Diethylamino) ethyl methacrylate (DEAEMA) was purchased from Aladdin Reagent (Shanghai) Co., Ltd, and it was passed through a column of activated neutral alumina to remove inhibitor before use. Silver nitrate (AgNO₃), sodium chloride (NaCl), Dimethyl sulfoxide (DMSO) was purchased from Sinopharm Chemical Reagent Co., Ltd. Ethylene glycol was purchased from Chinasun Specialty Products Co., Ltd. Deionized water was purified by using a ULUP water purification system with a resistivity of 18.25 mΩ cm⁻¹. The slides were purchased from Citotest Labware Manufacturing CO., LTD., models 10212450C (positively charged) and 188105 (negatively charged).

Characterization

¹H NMR (proton nuclear magnetic resonance spectrum) was collected with a Bruker nuclear magnetic resonance instrument (300 MHz) at room temperature. GPC (gel permeation chromatography, Agilent PL-GPC 50, Agilent Technologies Co., Ltd.) was performed using a dimethylformamide (DMF) solution containing 4.34 g L⁻¹ LiBr as a fluent. PMMA as standards. And the detector is the refractive index detector. Zeta potential and size were measured by DLS (dynamic light scattering, Zetasizer Nano ZS, Malvern Instruments Ltd.). SEM (scanning electron microscopy) images and EDX (energy dispersive X-ray spectroscopy) of micromotor were obtained on a Regulus SU8100 (Hitachi Inc.) scanning electron microscope.

Light source for polymerization

The xenon lamp is from Beijing China Education Au-light Co., Ltd., model CEL-HXF300. Light intensity was 70 mW cm⁻².
The synthesis of MAG

MAG was synthesized as previously reported.\textsuperscript{2} D-(+)-glucosamine hydrochloride (5 g, 20.24 mmol) and potassium carbonate (3.2 g, 23.16 mmol) were dissolved in 120 mL anhydrous methanol in a single neck round bottom flask using ice and ethanol to bathe. When the temperature of the solution drops to -15 °C, methacryloyl chloride (1.8 mL, 18.53 mmol) was added dropwise. After the dripping was completed, the reaction solution was kept at -15 °C for 30 minutes. The reaction lasted for 3 h and then products were purified by column chromatography (methanol : dichloromethane, 1 : 4) after solid in flask was removed by filtration. Finally, 3.44 g of white solid was obtained (yield: 59%). The following is the synthesis reaction formula of MAG and its $^1$H NMR spectrum (Fig. S1a and b).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{synthesis}
\caption{(a) Synthesis of MAG; (b) $^1$H NMR spectrum (D$_2$O) of MAG.}
\end{figure}

The synthesis of poly(MAG-co-DEAEMA)

The synthesis of poly(MAG-co-DEAEMA) via light-induced RAFT polymerization (Fig. S2a). MAG (0.4 g, 1.62 mmol), DEAEMA (65 μL, 0.32 mmol), CPDN (4.4 mg, 0.016 mmol) were dissolved by 1.2 mL DMSO inside 5 mL ampule with a magnetic stirring bar. The above solution was purged with argon for 20 min to deoxidize. Then the ampoule bottle was flame-sealed under argon atmosphere and placed under the light at room temperature. At the designed time, the ampoule bottle was opened and the mixture was dialyzed with a 3000 kDa dialysis bag for three days and then lyophilized. By $^1$H NMR (proton nuclear magnetic resonance spectrum), the ratio of MAG to DEAEMA is calculated to be 4.4 : 1 (Fig. S2b). The hydroxyl (3368 cm$^{-1}$), methyl (2968 cm$^{-1}$), methylene (2933 cm$^{-1}$), ester (1735 cm$^{-1}$) and amide (1644 and 1536 cm$^{-1}$) groups of the polymer were detected by FT-IR (Fig. S2c). The PDI (polymers dispersity index) of the polymers obtained by GPC (gel permeation chromatography) is 1.27 (Fig. S2d), and the obtained molecular weight is 25200 g mol$^{-1}$. 

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{polymerization}
\caption{The synthesis of poly(MAG-co-DEAEMA) via light-induced RAFT polymerization.}
\end{figure}
Glyco-micromotors were prepared as follows: 0.0415 g poly(MAG-co-DEAEMA) were dissolved in 5 mL of ethylene glycol. Then 0.0425 g of silver nitrate was added to the solution. After mixing the solution with a magnetic stirrer for 2 min, 1 mL of 0.0876 g mL\(^{-1}\) sodium chloride solution was slowly added to the solution. The solution was then heated to 150 °C. The reaction lasted at this temperature for 30 min. After the solution was cooled down to room temperature, the micromotors were then washed by centrifuging it down and resuspending it in deionized water two times. Finally, the micromotors were dispersed in 1.5 mL of ultrapure water and stored in the dark. The mass of the micromotor suspension after lyophilization was 28.8 mg (yield: 65%). In the micromotor suspension, the mass fraction of the micromotor was 1.88%.

The micromotor suspension was irradiated with UV light for 5 min. The micromotors were then washed twice with ultrapure water and lyophilized for FT-IR characterization. The following
is the FT-IR spectra of the micromotors before and after UV irradiation, and the polymer FT-IR spectrum is also given for comparison with the FT-IR spectrum of micromotors (Fig. S3).

**Fig. S3** (a) FT-IR spectrum of glycopolymer; (b) FT-IR spectrum of micromotors before and after UV irradiation.

**Effects of different HCl concentrations on the zeta potential and size of micromotors**

Prepare 1.5mL of 0, 1.3 mM, 2.7 mM, 5.3 mM, 8 mM, 10.7 mM, 200 mM, 400 mM, 600mM, 800mM and 1000 mM HCl solutions respectively, then add 40μL of micromotor suspension to each, and test the Zeta potential and size after standing for 5min.

**Specificity studies of glyco-micromotors**

Since the activation of Con A requires Ca$^{2+}$ and Mn$^{2+}$, an aqueous solution containing calcium chloride (0.11 mg mL$^{-1}$) and manganese chloride (0.002 mg mL$^{-1}$) is first prepared. 5 mg of Con A was added to 1 mL of calcium and manganese ion solution to prepare a 5 mg mL$^{-1}$ Con A solution. 5 mg of mannose was then dissolved in 2.5 mL of Con A solution. Then we took 250 μL of Con A and Con A/mannose solutions, respectively, and added 125 μL micromotor suspension to them. After standing at room temperature for 3h, observed with a microscope.

The interaction study of micromotors and E. coli is carried out according to the following steps: We chose E. coli MG1655 as the experimental bacteria. To ensure the uniformity of the bacterial shape and activity, the bacterial strain adopted single colony bacteria picked from the Luria-Bertani (LB) solid medium. The obtained single colony bacteria were grown in 1 mL LB liquid medium at 37 °C with shaking at speed of 190 rpm overnight and then washed three times with ultrapure water by centrifuging at 4000 rpm. The bacteria were resuspended in 1 mL ultrapure water for later use. 100 μL of H$_2$O or mannose aqueous solution (2 mg mL$^{-1}$) was added in a simple round sample cell made of a glass slide and a plastic ring fixed together with epoxy glue. The sample cell was cleaned in advance with ethanol and treated with a plasma cleaner. Then, 3 μL of micromotor suspension and 10 μL of bacterial suspension were added, mixed evenly, and allowed to stand for 15 minutes,
and observed with a microscope.

The interaction study of micromotors and S. aureus ATCC 12600 was same as E. coli, but the medium was change to Tryptone Soy Broth (TSB). Compared with E. coli, the binding rate of micromotors to S. aureus is much smaller (Fig. S4a-c).

![Fig. S4 Microscope images of (a) micromotors incubated with S. aureus, and (b) micromotors incubated with E. coli; (c) The binding rate of the micromotor to the S. aureus and E. coli. Scale bar: 10 μm.](image)

**Preparation of the complexes**

Bacterial culture as above. The bacteria were diluted to a cell density $\text{OD}_{600 \text{ nm}} = 0.05$ for later use.

The study of the individual behavior of the complexes was carried out according to the following steps: 100 μL of E. coli suspension ($\text{DO}_{600 \text{ nm}} = 0.02$) was added to the sample cell. Then 1.5 μL micromotor suspension was added to the sample cell. Mixed the mixture well with a pipette. Finally, the sample cell was placed in the dark for five minutes. Observe directly with a microscope after preparation of complexes. The following is the relationship between the speed of movement of the complex and the intensity of UV light.

![Fig. S5 The effect of UV intensity on the speed of the complex during individual motion.](image)

When studying aggregation behavior, we used more concentrated E. coli suspensions ($\text{OD}_{600 \text{ nm}} = 0.05$), and more micromotor suspensions (5 μL). Other conditions remain unchanged.

**Microscopy Observation and Image Processing**

The movement of the complexes was observed by a Basler ACE camera fitted on an Olympus IX73 microscope using a 40X objective. UV light was focused onto the complexes through the microscope objective from below. The maximum UV light intensity was 35 mW cm$^{-2}$. Image
analysis was done via ImageJ and python. The light field strong above and weak below were realized by blocking half of the light path.

References: