Supplementary Information

NIR-Triggered Drug Release from Switchable Rotaxane-Functionalized Silica-Covered Au Nanorods

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Experiment Section

1. Materials and equipment

All the chemicals were purchased from Sigma-Aldrich and used without any purification. FT-IR spectra were recorded on a Perkin-Elmer 1760X FT-IR spectrometer with the sample powder diluted in KBr (1%). BET surface areas were determined using a Micromeritics ASAP 2020M automated sorption analyzer. The transmission electron microscopy (TEM) images were observed using a JEOL 2010. UV-vis analyses were performed on a Shimadzu UV-3600 UV-Vis-NIR spectrophotometer (1-mm quartz cell used). Fluorescent spectra were recorded on a Shimadzu RF-5301 spectrofluorophotometer.

2. Preparation of mesoporous silica covered Au nanorods (Au@MSN)

The Au nanorods were synthesized according to previous study with some modifications[1]. Generally, the ultra-small Au seeds were prepared by the reduction of $HAuCl_4$ using $NaBH_4$ in aqueous environment. Aqueous cetyltrimethyl ammonium bromide (CTAB) solution (0.2M, 10 mL) was first mixed with $HAuCl_4$ solution (m/V 1%, 100 μL). Then, ice-cold $NaBH_4$ aqueous solution (0.01 M, 0.6 mL) was added to the above mixture. The ultra-small Au seeds were formed immediately upon the addition of $NaBH_4$ solution. The Au seeds were always used within 3 h after the preparation.

The growth solution for Au nanorods was a mixture of CTAB solution (0.1 M, 100 mL), $HAuCl_4$ solution (0.01 M, 5 mL), $AgNO_3$ solution (10 mM, 1 mL), H_2SO_4 solution (0.5 M, 2 mL) and ascorbic acid solution (0.1 M, 800 μL) added sequentially. The growth was initiated by adding above seed solution (240 μL). The nanorod growth was carried out for 6 h and the temperature was kept at 30 °C. The Au nanorods prepared were first washed with deionized water for several times to remove the excessive CTAB and remaining reactants. The Au nanorods were extracted through centrifugation and then concentrated to 10 mg Au/mL.

The mesoporous silica coating was achieved *via* a template method. Firstly, the concentrated Au nanorod solution (1 mL) was re-dispersed in aqueous CTAB solution (0.01 M, 100 mL), and the mixture was gently stirred for about 15 min. Ammonia water (0.01M, 1 mL) was subsequently added into the mixture solution in order to adjust the solution pH to be slightly basic, followed by the injections of TEOS (30 μL each) for three times with 30 min interval. The temperature of the mixture was kept at 30°C. The reaction was carried out about 24 h. The silica coated Au nanorods were then extracted *via* centrifugation and washed with ethanol for 3 times.

The CTAB template was removed by using ethanol solution of hydrochloride. To be specific, silica coated Au nanorods (60 mg) were dispersed in ethanol (40 mL) containing hydrochloric acid (m/V 35%, 5 mL), and the mixture was stirred at 40°C. After 24 h of reaction, mesoporous silica coated Au nanorods were collected through centrifugation. The extracted sample was anhydrated under vacuum and the product was denoted as Au@MSN.

3. Synthesis of N-(3-(triethoxysilyl) propyl) propiolamide

N-(3-(triethoxysilyl) propyl) propiolamide was prepared according to a previous report[2]. *N*,*N*-Dicyclohexylcarbodiimide (2.46 g, 12.0 mmol) was added into a mixed solution of 3-aminopropyltriethoxysilane (2.40 g, 10.9 mmol) and propiolic acid (0.87 g, 11.9 mmol) in dichloromethane (15 mL). The mixture was kept stirring at room temperature for 1 h, and then roughly separated using a filter paper. Evaporation of excessive solvent from the filter liquor was achieved by co-evaporation with toluene (40 mL). The co-evaporation was repeated 3 times, and the solution was diluted with toluene to 20 mL.

4. Grafting N-(3-(triethoxysilyl) propyl) propiolamide onto Au@MNS surface (Au@MSN-Alkyne)

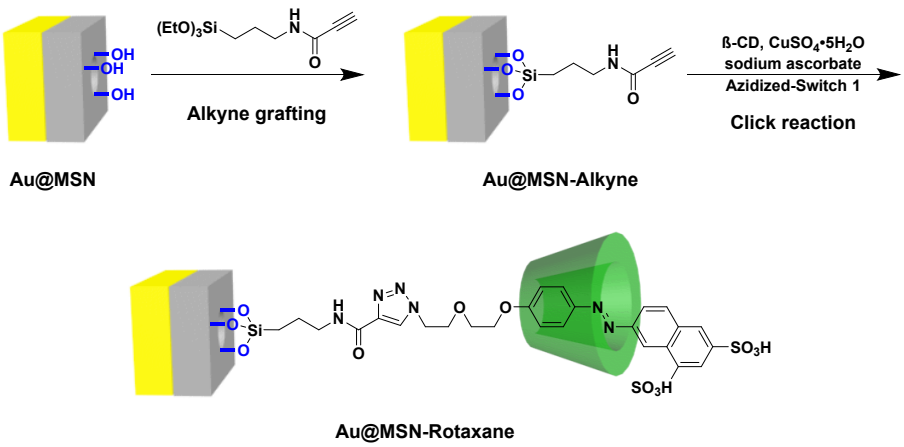
The anhydrous Au@MSN was homogeneously suspended in toluene (10 mg powder in 20 mL anhydrous toluene). N-(3-(triethoxysilyl) propyl) propiolamide solution (1 mL) was

subsequently added. The mixture was refluxed at 120°C for 24 h. After that, the product was extracted *via* centrifugation. The precipitate was washed first by acetone and then deionized water for several times, followed by anhydration under vacuum at room temperature. The product was denoted as Au@MSN-Alkyne.

5. Synthesis of 7-((4-(2-(2-azidoethoxy) ethoxy) phenyl) diazenyl) naphthalene-1,3-disulfonic acid and conjugation of photo-responsive rotaxane onto Au@MSN-Alkyne

The synthesis of the azobenzene moiety, 7-((4-(2-(2-azidoethoxy) ethoxy) phenyl) diazenyl) naphthalene-1,3-disulfonic acid, was achieved by following a route provided in a previous paper (Scheme S1)[3]. The azobenzene moiety (150 mg, 0.27 mmol) and α-cyclodextrin (525 mg, 0.54 mmol) were then dissolved in H_2O (10 ml), which was stirred at room temperature under N_2 protection for 2 h to give the complex, denoted as Azidized-Switch 1.

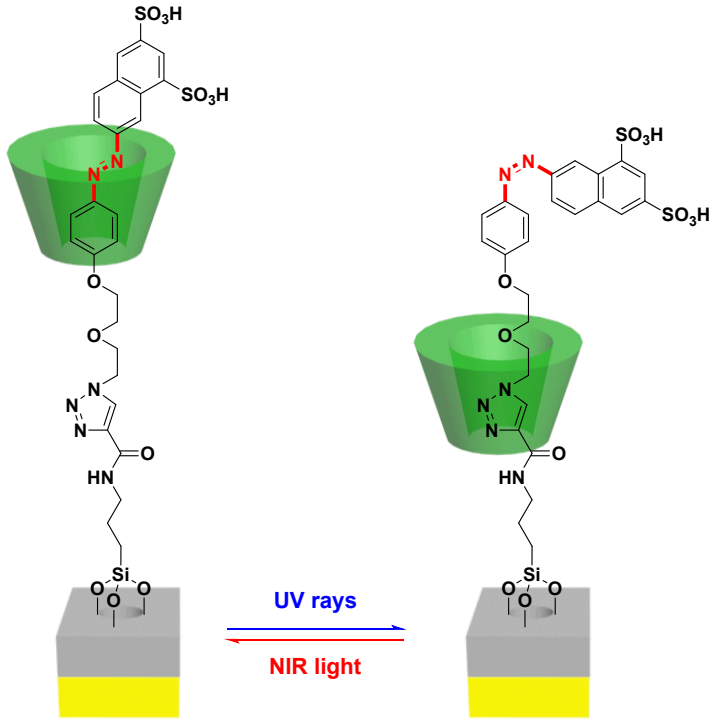
A solution of Au@MSN-Alkyne (20 mg), $CuSO_4 \cdot 5H_2O$ (3.4 mg), and sodium ascorbate (3.4 mg) in H_2O (5 mL) were added to the above complex solution. The mixture solution was then stirred at room temperature for 3 days. The product was extracted *via* centrifugation, and then washed by deionized water for several times to afford the final product, denoted as Au@MSN-Rotaxane (Scheme S1).



Scheme S1. Synthetic procedure for the azobenzene derivative conjugates.

6. Characterizing the conformation transition of Au@MSN-Rotaxane

Au@MSN-Rotaxane (100 μg) was dispersed in deionized water, which was stored at 35°C for 10 min. The UV-vis absorbance was subsequently measured and the obtained absorption spectrum was denoted as the "on" state. The sample was cooled down to room temperature and then irritated with UV light at 365 nm for 15 min. The UV-vis spectrum was measured again, which was denoted as the "off" state. The same cuvette was subsequently illuminated with NIR laser (808 nm, 200mW/cm^2) for 30 min before the UV-vis spectrum was measured (Scheme S2). The UV-NIR illumination processes were repeated for 5 cycles using the same cuvette and sample solution in order to reduce the interference of other potential factors.



Scheme S2. Photo-induced conformation transition of Au@MSN-Rotaxane

7. Photothermal effect of Au@MSN-Rotaxane

Au@MSN-Rotaxane (500 μg) was dispersed in deionized water (0.5 mL), which was transferred into a cuvette. To achieve the maximized conversion efficiency, the cuvette was wrapped in tin foil. Temperature reading of the solution was recorded by a digital

thermometer. The lens module of the NIR laser was located right above the cuvette and the calculated power density of the NIR illumination was 1.5W/cm^2. Same volume of deionized water was used as the experiment control, which was also continuously illuminated with NIR laser under the same power density. Initial temperature of all the sample solutions was 25°C. In the experiment, the sample solution was continuously illuminated with NIR laser under the same power density. The duration of the NIR illumination was 1 h and the temperature of the sample solution was recorded when the illumination was complete.

8. Drug loading and release profile measurement

FITC, a widely used fluorophore, was used as the model drug to be loaded into Au@MSN-Rotaxane *via* diffusion in aqueous solution at 40°C under dark environment for 1 day. FITC-loaded Au@MSN-Rotaxane was then irritated with UV light at 365 nm for 15 min, where *trans*-to-*cis* isomerization of the azobenzene-based rotaxane in Au@MSN-Rotaxane enabled the closure of the mesopores. FITC-loaded Au@MSN-Rotaxane was extracted through centrifugation, and then washed with deionized water for several times to remove free FITC attached on the particle surface. For the release test, each cuvette contained 20 μg of FITC-loaded Au@MSN-Rotaxane, and deionized water was added to make the total volume of 1mL. Three same sample groups were prepared. The release behavior of FITC-loaded Au@MSN-Rotaxane against heat and NIR irradiation was monitored by the spectrofluorophotometer. The excitation and emission wavelengths were 495 nm and 519 nm, respectively. A blank sample where no specific trigger was applied (25°C, placed in dark environment) was used as the control group. For the temperature induced release, the temperature of the sample solution was kept at 25°C for the first 5 min and subsequently heated to 45°C using a digital heat source. For the NIR triggered release, the sample solution was illuminated with NIR laser continuously (power output: 200mW/cm^2). The cuvette was wrapped with tin foil for maximizing the NIR absorption. For all the three samples, the

fluorescent intensity readings were taken after 5 min, 11 min, 19 min, 45 min, 100 min and 180 min, respectively. The release tests under each condition were repeated for 3 times to eliminate accidental error.

9. *In vivo* evaluation using zebrafish embryo models

Doxorubicin (DOX) as one of the typical clinical anticancer drug was used as the cargo for delivery and fluorescent indicator. Au@MSN-Rotaxane (40 mg) was dissolved in aqueous DOX solution (1mg/mL, 10 mL), and the mixture was stirred at 40°C in dark environment for 1 day to allow for maximized loading amount. DOX-loaded Au@MSN-Rotaxane was also extracted through centrifugation. It was illuminated with UV light for 15 min and subsequently stored in a sealed container out of light at 25°C. For every 5-day old zebrafish embryo, a dose of concentrated DOX-loaded Au@MSN-Rotaxane solution (40mg/mL, 5μL) was injected into its brain ventricle. Each mounted zebrafish embryo was imaged under bright field with HeNe 543 nm laser attached to a 560/615 nm emission band-pass filter, which were used to simultaneously detect optically opaque gold nanorods and the intrinsic red fluorescent of the DOX cargo. For the release test in the zebrafish embryo models, the particle-injected embryos were illuminated with NIR light (power density: 1.5 W/ cm^2) for 1 h. The experimental samples were then incubated together with the controls in a dark environment at 25°C for another 2 days before imaging. This process was to evaluate the long-term effect *in vivo*. For the study of the drug-release kinetic characteristics, the two experimental groups were illuminated with NIR light for 10 min and 5 min respectively, one day after the injection under the same power density (1.5W/cm^2). The embryos were then immobilized and mounted, and simultaneously imaged for 2 h after the NIR illumination. In another experimental group, the injected embryos were incubated under the physiological temperature of 37°C for 3h and subsequently imaged. The image size and corresponding positions in the imaged area were kept the same, and a fixed line where the terminal position

was demarcated by an arrowhead was first drawn across the bright field image. Bright field intensity profile, detecting the distance occupied by the opaque Au@MSN-Rotaxane, with the minimum gray value along the demarcated line was measured and depicted. The DOX intensity profile was subsequently examined across the same position and length using Image J. A red serrated box was drawn to delimit the region that corresponded to the DOX trapped in the mesoporous silica layer of the Au nanorods. Equal distance was selected and compared across all samples and control group.

Experimental results

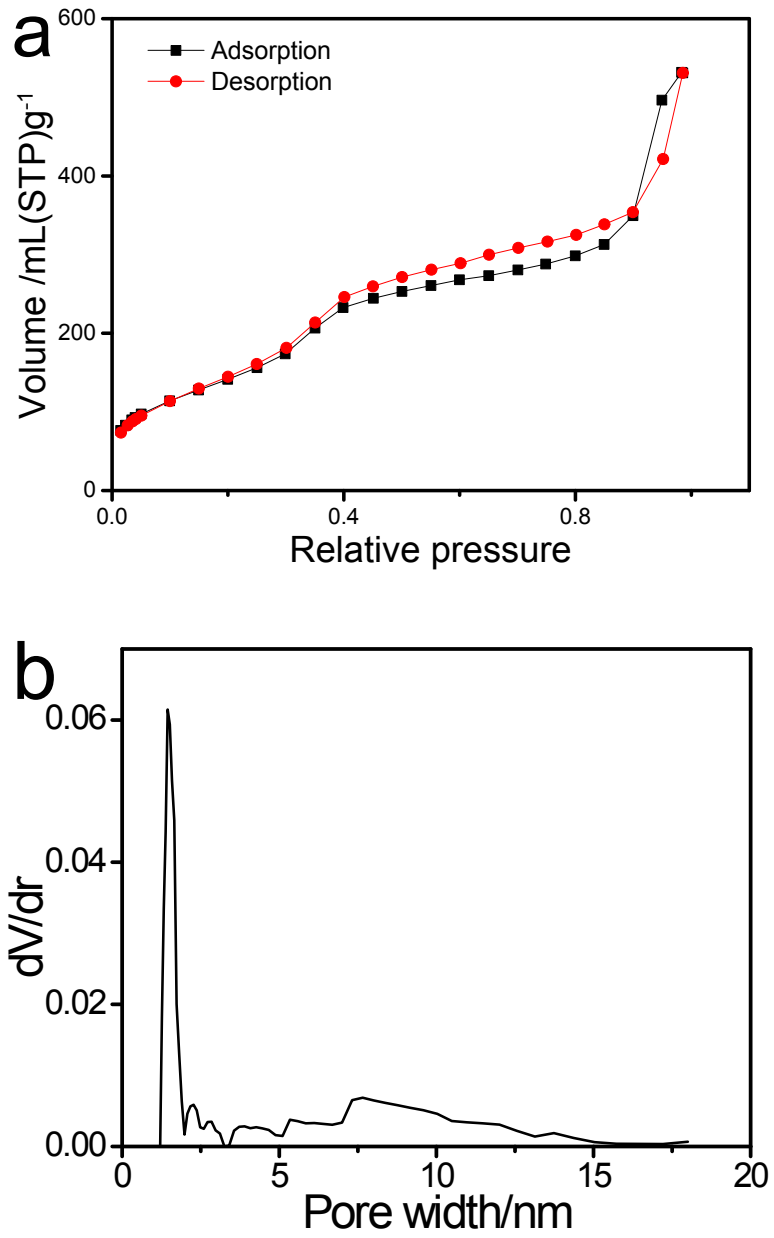


Figure S1. a) BET isotherms and b) the pore size distribution of Au@MSN.

The N_2 adsorption/desorption isotherms and the corresponding pore size distribution curves (Figure S1) indicate detailed porous properties of Au@MSN. The Au@MSN has a total surface area of 535.110m² g^{-1}, a pore volume of 0.8215 cm^3/g and an average pore diameter of 1.45 nm.

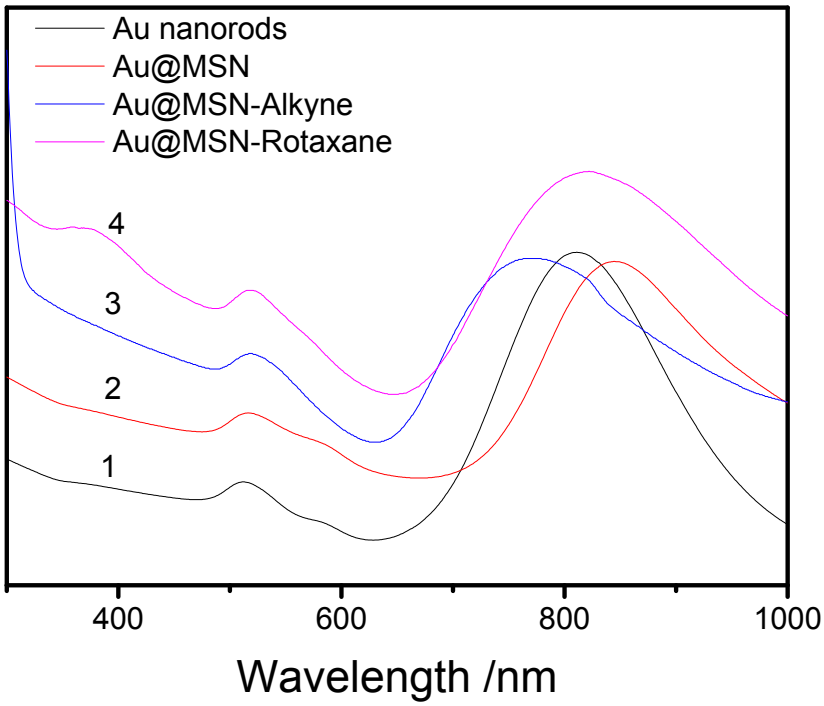


Figure S2. UV-vis spectra showing the evolution of the drug delivery system through different modification stages. 1: Au nanorods, 2: Au@MSN, 3: Au@MSN-Alkyne, and 4: Au@MSN-Rotaxane.

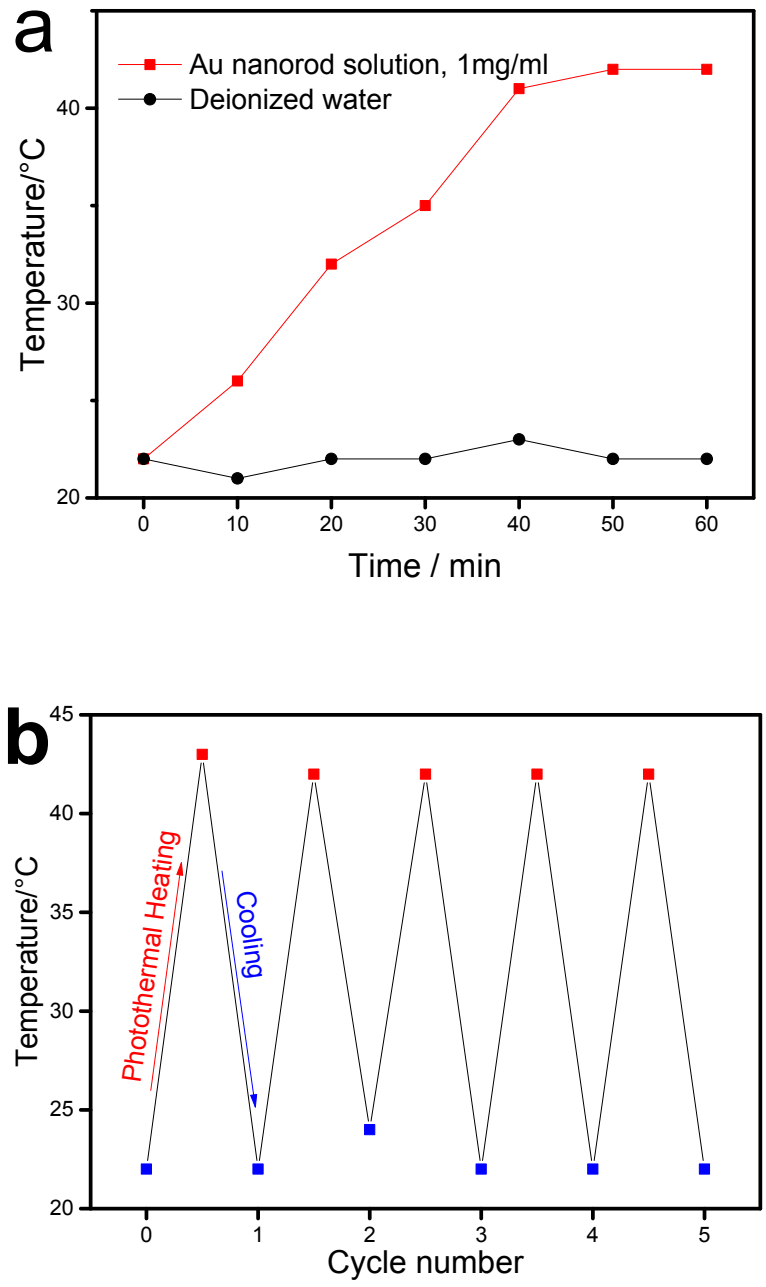


Figure S3. Photothermal effect of Au@MSN-Rotaxane. a) Temperature increase when illuminated with an NIR laser. b) The temperature of Au@MSN-Rotaxane solution after 1h NIR laser illumination, plotted against the number of heating-cooling cycles.

As shown in Figure S3a, the temperature of Au@MSN-Rotaxane solution increased rapidly when illuminated with an NIR laser, while the temperature change of deionized water under the same illumination condition was almost negligible. The difference confirmed the potent photothermal efficiency of Au@MSN-Rotaxane. The thermal-stability of Au@MSN-Rotaxane was demonstrated in Figure S3b, as it showed that the photothermal effect of Au@MSN-Rotaxane was still retained after 5 rounds of heating-cooling cycles.

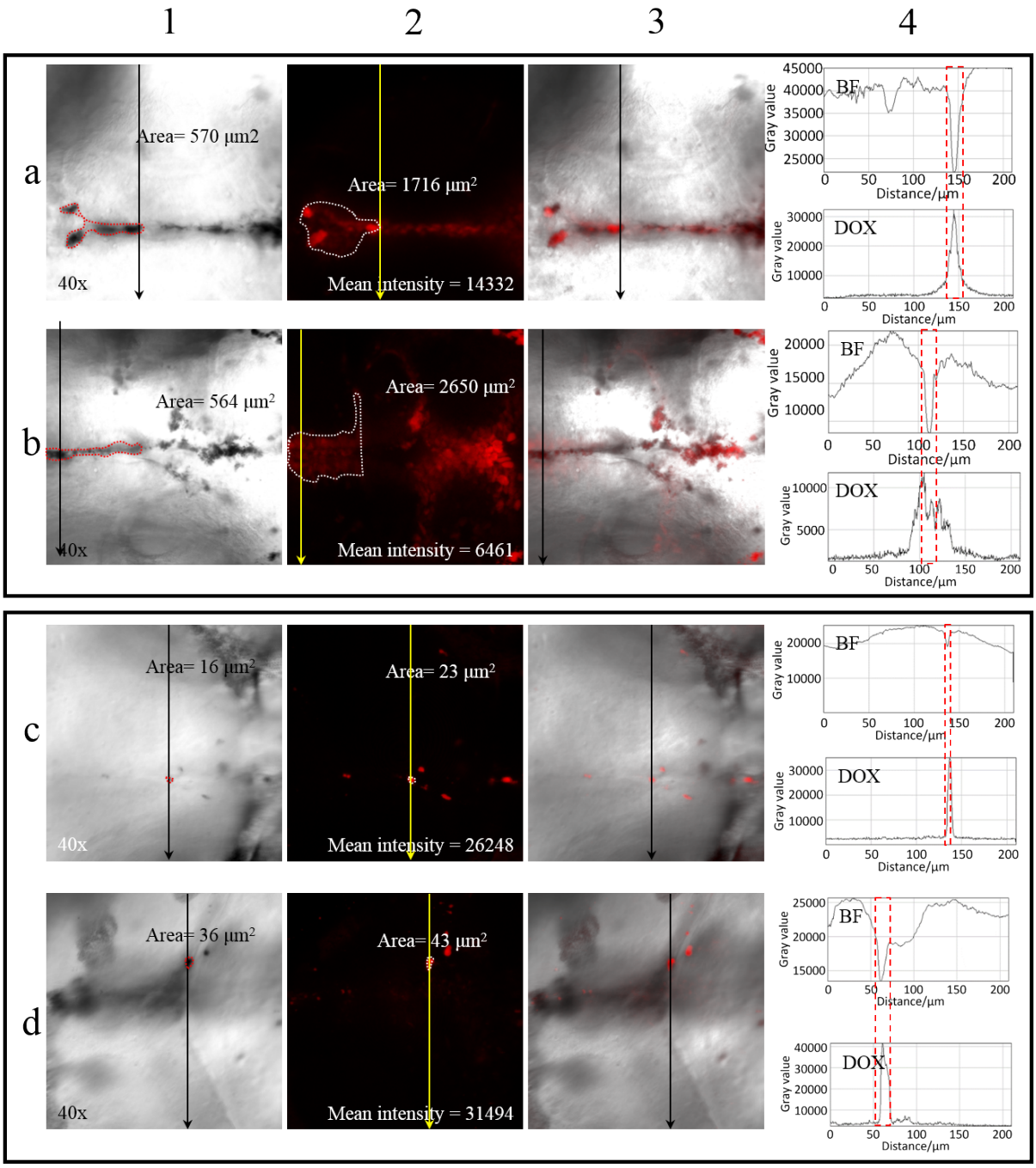


Figure S4. Confocal images and intensity plot of gray value showing the DOX release from the injected DOX-loaded Au@MSN-Rotaxane at 25°C. Each sample group was marked by a box. Sample a and c are the blank controls in each experimental group. b) Embryo illuminated with NIR for 10 min. d) Embryo illuminated with NIR for 5 min. 1) Images taken under bright field. 2) DOX fluorescence, 3) Merged image of 1 and 2, and 4) Intensity plot of the gray value across the arrow.

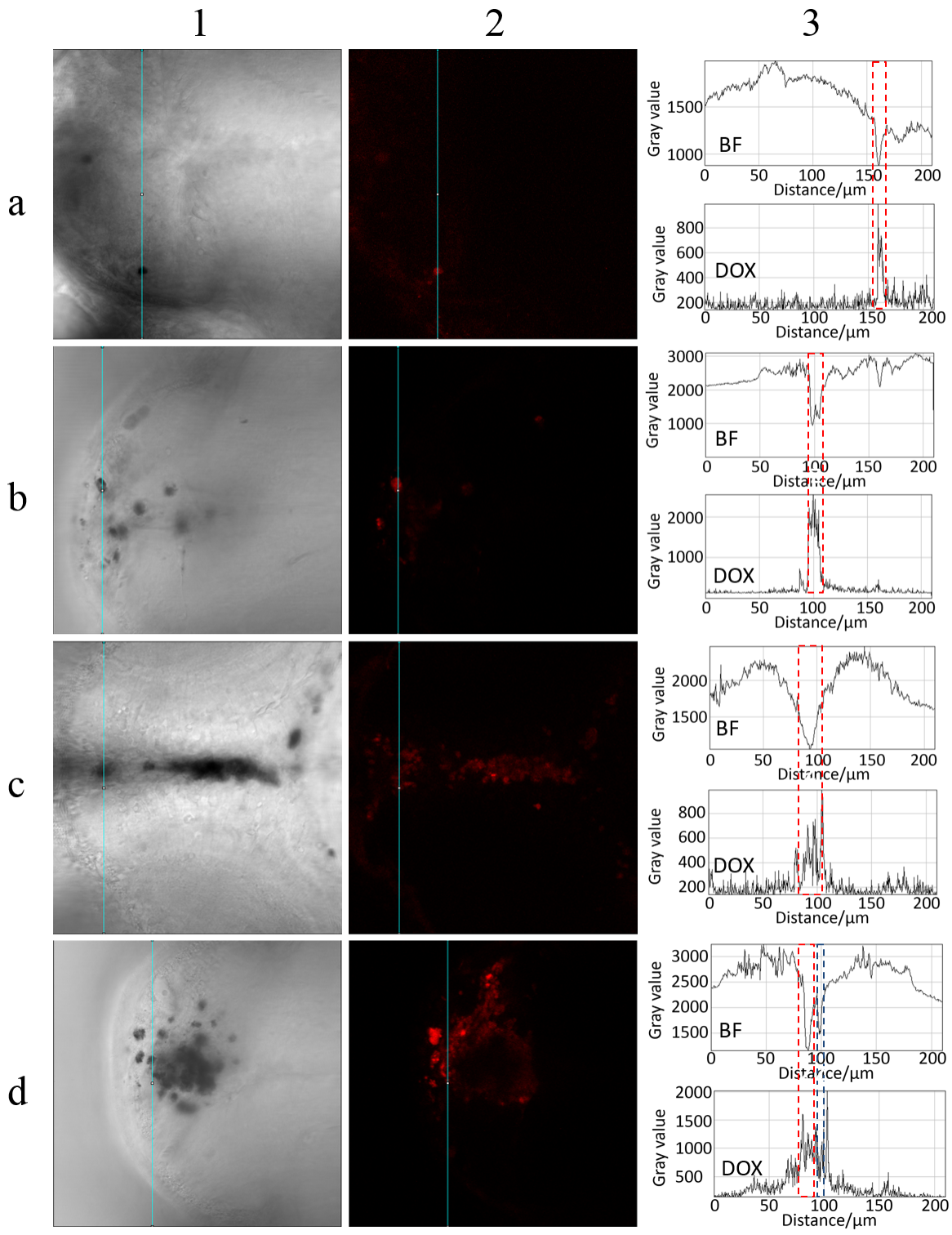


Figure S5. Confocal images and the gray value intensity plots showing the DOX release at 37°C from the DOX-loaded Au@Silica-Rotaxane in zebrafish embryos. Top-down: a) Un-illuminated sample, b) NIR illumination for 10 min, c) NIR illumination for 20 min, and d) NIR illumination for 30 min. Left-right: 1) Images taken under bright field, 2) DOX fluorescence, and 3) intensity plot of the gray value across the blue line.

At 37°C, some background noises (around 30%) were observed in the control group due to the particle dispersion during the local injection. However, it can be noted that the major red DOX fluorescence still overlapped with the DOX-loaded Au@Silica-Rotaxane. The distance of DOX fluorescence spreading increased significantly after extending the NIR illumination time (from 10 to 30 min), indicating that the mode of release is indeed NIR-triggered release. Overall, the *in vivo* experiment conducted at 37°C is consistent with the conclusion that the NIR light can effectively trigger the drug release from the nanocarrier, and moreover, the released drug amount correlates positively with the illumination duration.

References:

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