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Supporting Information

Redox-Sensitive Stomatocyte Nanomotors: Destruction and Drug Release in the Presence of Glutathione

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Author Contributions

Y.T. Conceptualization: Equal; Formal analysis: Lead; Investigation: Lead; Methodology: Lead; Writing – original draft: Equal; Writing – review & editing: Equal
F.P. Data curation: Supporting; Formal analysis: Supporting; Investigation: Equal; Visualization: Equal; Writing – review & editing: Equal
P.W. Formal analysis: Supporting; Methodology: Supporting; Validation: Supporting; Writing – review & editing: Supporting
D.W. Conceptualization: Lead; Formal analysis: Equal; Funding acquisition: Lead; Investigation: Equal; Methodology: Equal; Project administration: Lead; Resources: Lead; Supervision: Lead; Validation: Lead; Writing – original draft: Lead; Writing – review & editing: Equal.

Supplementary Information

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1. Materials

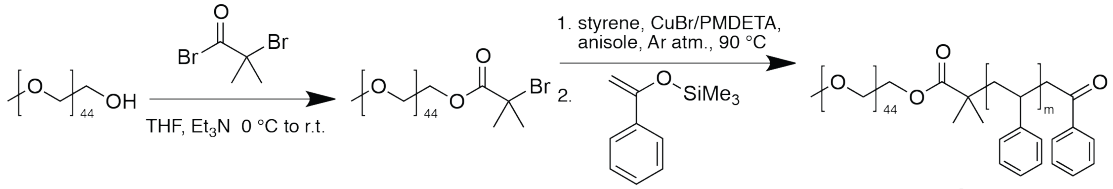
Unless stated otherwise, all reagents and chemicals were used without further purification. Styrene (Sigma-Aldrich) was distilled before polymerization to remove the inhibitor. CuBr purchased from Sigma-Aldrich for Atom Transfer Radical Polymerization (ATRP) was washed with acetic acid and followed by methanol for three times and subsequently stored under argon. Tetrahydrofuran (THF) for the ATRP reaction was freshly distilled under argon from sodium/benzophenone. MilliQ water obtained with a MilliQ QPOD purification system (18.2 MΩ) was used for self-assembly and dialysis of polymersomes/stomatocytes. Spectra/Por® Dialysis Membrane (MWCO: 12-14,000 Da) was used for dialysis of polymersomes/stomatocytes. Polyvinyl pyrrolidone (PVP, Mn 10 kg/mol), poly(ethylene glycol) methyl ether (Mn 2 kg/mol), L (+) ascorbic acid, magnesium sulfate, sodium chloride, ethylenediaminetetraacetic acid (EDTA), potassium tetrachloroplatinate (II) (K_2PtCl_4), 1-phenyl-1-trimethylsiloxyethene, 3,3'-dithiodipropionic acid (DTDP), α-bromoisobutyryl bromide, 4-(dimethylamino)pyridine (DMAP), N,N'-dicyclohexylcarbodiimide (DCC), 2-hydroxyethyl 2-bromoisobutyrate, chloroform-d ($CDCl_3$) and N,N,N',N'',N''-pentamethyldiethylenetriamine (PMDETA) were purchased from Sigma-Aldrich. THF and anisole were obtained from Acros. MeOH, triethylamine and hydrogen peroxide were purchased from J.T. Baker. Diethyl ether (Carlo erba Reagents), 1,4-dioxane (Biosolve BV) and dichloromethane (CH_2Cl_2, Fisher Chemical) were used as received.

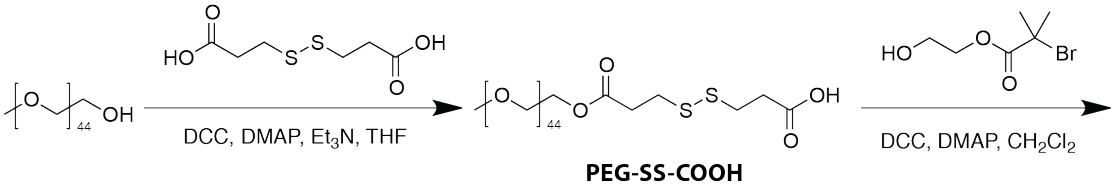
2. Instruments

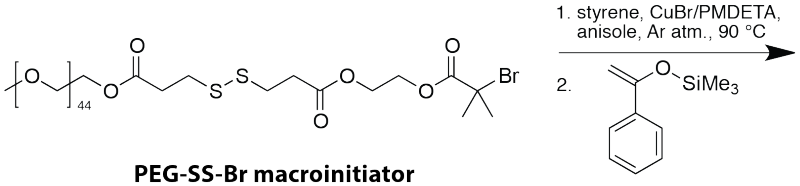
Routine NMR spectra were recorded on a Varian Inova 400 spectrometer with $CDCl_3$ as a solvent. Malvern Zetasizer Nano ZS was used for dynamic light scattering (DLS)

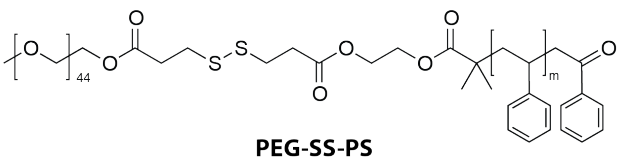
analysis with the following settings: temperature 25 °C, He-Ne laser wavelength 633 nm and detector angle 173°. For transmission electron microscopy, a JEOL 1010 Transmission Electron Microscope with MegaView Soft Imaging camera at an acceleration voltage of 60 kV was used. JEOL 6330 Cryo Field Emission Scanning Electron Microscope was used for acquiring SEM images. Diffusion measurements were performed at 298 K on a Bruker Avance III 500 MHz spectrometer equipped with a BBFO probe. The maximum z-gradient for the probe is 53.5 G/cm. Diffusion measurements were calibrated to pure methanol and ethylene glycol. The pulse sequence incorporated delays for eddy currents to dissipate and bipolar gradients for encoding and decoding diffusion information. Nanoparticle tracking analysis (NTA) of stomatocyte nanomotors was performed on a NanoSight NS500.

3. Synthetic procedures, self-assembly and characterizations









Supplementary Scheme 1 Synthetic route for the block copolymer PEG-*b*-PS and redox-sensitive PEG-SS-PS.

3.1. Synthesis of α-methoxy-poly(ethylene glycol)$_{44}$ ATRP macromolecular initiator (1)

Poly(ethylene glycol) methyl ether (5.00 g, 2.50 mmol) was dried by co-evaporation with toluene. The polymer was dissolved in freshly distilled THF in a flamed-dried Schlenk flask. After adding triethylamine (1.04 mL, 7.50 mmol), the mixture was cooled to 0 °C. α-bromoisobutyryl bromide (616 μL, 5.00 mmol) was added dropwise. After addition, the resulting solution was stirred for 24h while slowly warming to room temperature. After the reaction, the white precipitate was filtered off and the solution was concentrated. The polymer was precipitated in ice-cold diethyl ether (3x) and dried under vacuum overnight. The polymer was characterized by ^{1}H-NMR in $CDCl_3$.

^{1}H-NMR (400 MHZ, $CDCl_3$) δ: 4.33 (t, $CH_2CH_2OC(O)C(CH_3)_2Br$), 3.76 (t, $CH_2CH_2OC(O)C(CH_3)_2Br$), 3.65 (br. s, PEG backbone), 3.55 (m, CH_3OCH_2), 3.38 (s, CH_3OCH_2), 1.94 (s, $C(CH_3)_2Br$) ppm.

3.2. Synthesis of poly(ethylene glycol)-*b*-polystyrene (2)

The Schlenk tube with CuBr (45 mg, 0.32 mmol) was evacuated for 15 min and refilled with Ar for three times. PMDETA (66 μL, 0.32 mmol) in anisole (0.5 mL) was added, followed by 15 min vigorously stirring. Styrene (5 mL, 43.6 mmol) in anisole (0.5 mL) was added via a syringe and degassed for 15 min. After cooling the mixture to 0 °C, PEG-initiator (215 mg, 0.1 mmol) dissolved in anisole (0.5 mL) was injected and the solution was degassed for another 15 min. The Schlenk tube was transferred into an oil bath at 90 °C. ^{1}H-NMR was used for monitoring the reaction process. Upon attainment of the required molecular weight, 1-phenyl-1-

trimethylsiloxyethene (1.91 mL, 9.28 mmol) was added to quench the polymerization. The reaction was terminated by cooling to room temperature after stirring for 2h. The solution was diluted with CH_2Cl_2 and extracted with an aqueous EDTA solution (65 mM). The organic layer was collected and dried with $MgSO_4$ and concentrated. The polymer was obtained after precipitation in MeOH (3x) and dried under vacuum overnight. The polymer was characterized by ^{1}H-NMR in $CDCl_3$.

^{1}H-NMR (400 MHZ, $CDCl_3$) δ: 7.20-6.30 (br. s, PS arom.), 3.64 (br. s, PEG backbone), 3.38 (s, CH_3OCH_2), 2.30-1.20 (br. s, PS backbone), 0.90 (br. m, $C(O)C(CH_3)_2CH_2$) ppm.

3.3. Synthesis of PEG-SS-COOH (3)

PEG-SS-COOH was synthesized according to the literature.[1] Poly(ethylene glycol) methyl ether (8.00 g, 4 mmol) was dried by co-evaporation with toluene for three times. Then the polymer, DTDP (1.57 g, 7.5 mmol), DCC (1.60 g, 8 mmol), DMAP (0.48 g, 4 mmol) and triethylamine (0.41 g, 4 mmol) were dissolved in freshly distilled THF in a flamed-dried Schlenk flask. The resulting solution was stirred for 2 days. After the reaction, the white precipitate dicyclohexylurea (DCU) was filtered off and the solution was concentrated. The polymer was precipitated in ice-cold diethyl ether (3x) and dried under vacuum overnight. The polymer was characterized by ^{1}H-NMR in $CDCl_3$.

^{1}H-NMR (400 MHZ, $CDCl_3$) δ: 3.66 (s, CH_2CH_2O), 3.40 (s, OCH_3), 2.91-2.99 (m, $CH_2CH_2SSCH_2CH_2$), 2.73-2.81 (m, $CH_2CH_2SSCH_2CH_2$) ppm.

3.4. Synthesis of PEG-SS-Br ATRP macroinitiator (4)

PEG-SS-Br macroinitiator was synthesized according to the literature.[1] PEG-SS-COOH (2.10 g, 1 mmol), DMAP (122 mg, 1 mmol), DCC (413 mg, 2 mmol) and 2-hydroxyethyl 2-bromoisobutyrate (422 mg, 2 mmol) were dissolved in fresh-distilled

CH_2Cl_2. The mixture was stirred at room temperature for 2 days. Byproduct DCU was removed by filtration. The filtrate was concentrated and precipitated in ice-cold diethyl ether (3x) and dried under vacuum overnight. The polymer was characterized by ^{1}H-NMR in $CDCl_3$.

^{1}H-NMR (400 MHZ, $CDCl_3$) δ: 4.38 (d, $COOCH_2CH_2COO$), 3.65 (s, CH_2CH_2O), 3.38 (s, OCH_3), 2.91-2.94 (t, $CH_2CH_2SSCH_2CH_2$), 2.75-2.79 (t, $CH_2CH_2SSCH_2CH_2$), 1.94 (s, $C(CH_3)_2Br$)

3.5. Synthesis of PEG-SS-PS (5)

The Schlenk tube with CuBr (45 mg, 0.32 mmol) was evacuated for 15 min and refilled with Ar for three times. PMDETA (66 μL, 0.32 mmol) in anisole (0.5 mL) was added, followed by 15 min vigorously stirring. Styrene (5 mL, 43.6 mmol) in anisole (0.5 mL) was added via a syringe and degased for 15 min. After cooling the mixture to 0 °C, PEG-SS-Br macroinitiator (230 mg, 0.1 mmol) dissolved in anisole (0.5 mL) was injected and the solution was degassed for another 15 min. The Schlenk tube was transferred into an oil bath at 90 °C. ^{1}H-NMR was used for monitoring the reaction process. Upon attainment of the required molecular weight, 1-phenyl-1-trimethylsiloxyethene (1.91 mL, 9.28 mmol) was added to quench the polymerization. The reaction was terminated by cooling to room temperature after stirring for 2h. The solution was diluted with CH_2Cl_2 and extracted with an aqueous EDTA solution (65 mM). The organic layer was collected and dried with $MgSO_4$ and concentrated. The polymer was obtained after precipitation in MeOH (3x) and dried under vacuum overnight. The polymer was characterized by ^{1}H-NMR in $CDCl_3$.

^{1}H-NMR (400 MHZ, $CDCl_3$) δ: 7.20-6.30 (br. s, PS arom.), 3.64 (br. s, PEG backbone), 3.38 (s, CH_3OCH_2), 2.30-1.20 (br. s, PS backbone), 0.90 (br. m, $C(O)C(CH_3)_2CH_2$) ppm.

3.6. Preparation of PtNPs with PVP coating

4 mL K_2PtCl_4 solution (20 mM) was added into 40 mg PVP, followed by 48 hours stirring. After that, 35 mg *L* (+) ascorbic acid in 1 mL of MilliQ water was added into the solution. The resulting solution was sonicated (VWR Ultrasonic Cleaner Model 75D) at room temperature for 1 h.

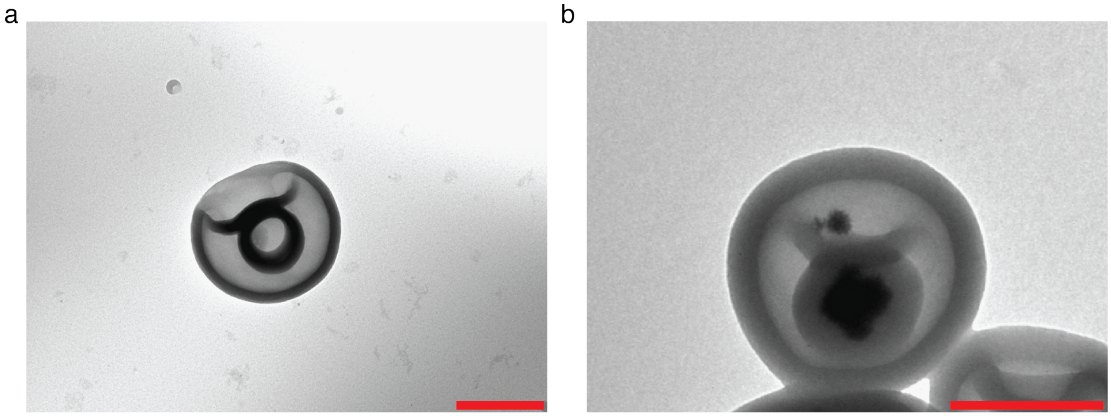
3.7. Self-assembly of stomatocyte or redox-sensitive stomatocyte

10 mg PEG-*b*-PS (or 10 mg PEG-SS-PS) was fully dissolved in 1 mL mixture of THF/dioxane (4:1, v/v). 1 mL of MilliQ water was slowly added into the solution by a syringe pump at a rate of 1 mL/h. After vigorous dialysis for at least 48 hours, regular stomatocytes or redox-sensitive stomatocytes were obtained.

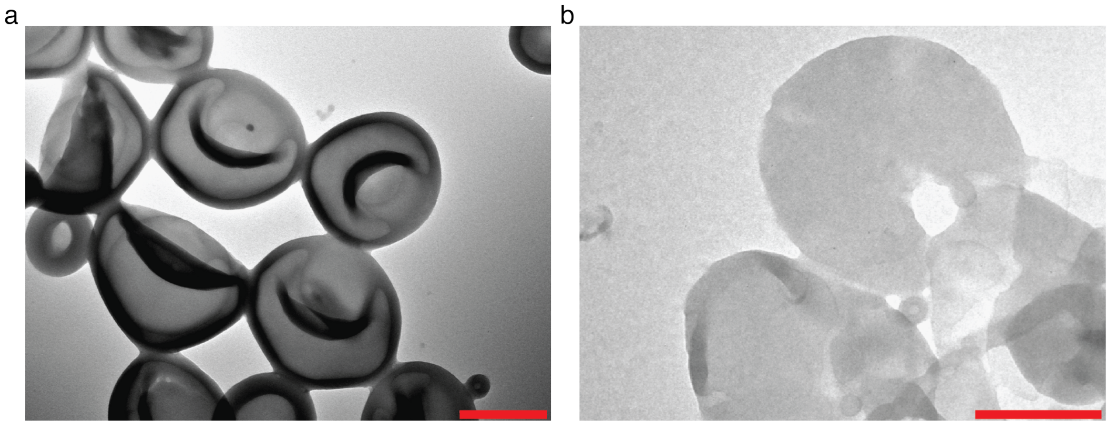
3.8. Self-assembly of stomatocyte nanomotor or redox-sensitive stomatocyte nanomotor

10 mg PEG-*b*-PS (or 10 mg PEG-SS-PS) was fully dissolved in 1 mL mixture of THF/dioxane (4:1, v/v). 0.35 mL of MilliQ water was slowly added by a syringe pump at a rate of 1 mL/h, followed by addition of preformed PtNPs solution (0.65 mL) also at a rate of 1 mL/h. After dialysis for at least 48 hours, stomatocyte nanomotors or redox-sensitive stomatocyte nanomotors were obtained.

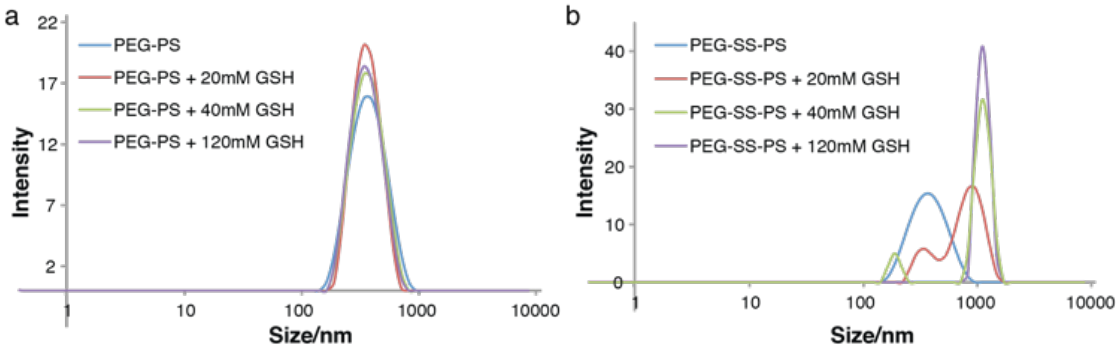
4. Supplementary Figures and Tables



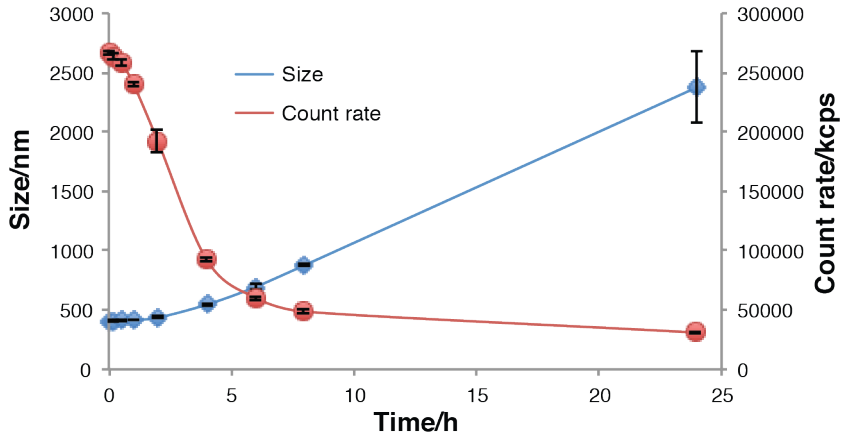
Supplementary Figure 1. a. TEM image of non-sensitive stomatocyte; b. TEM image of non-sensitive stomatocyte nanomotor. (Scale bar: 200 nm)



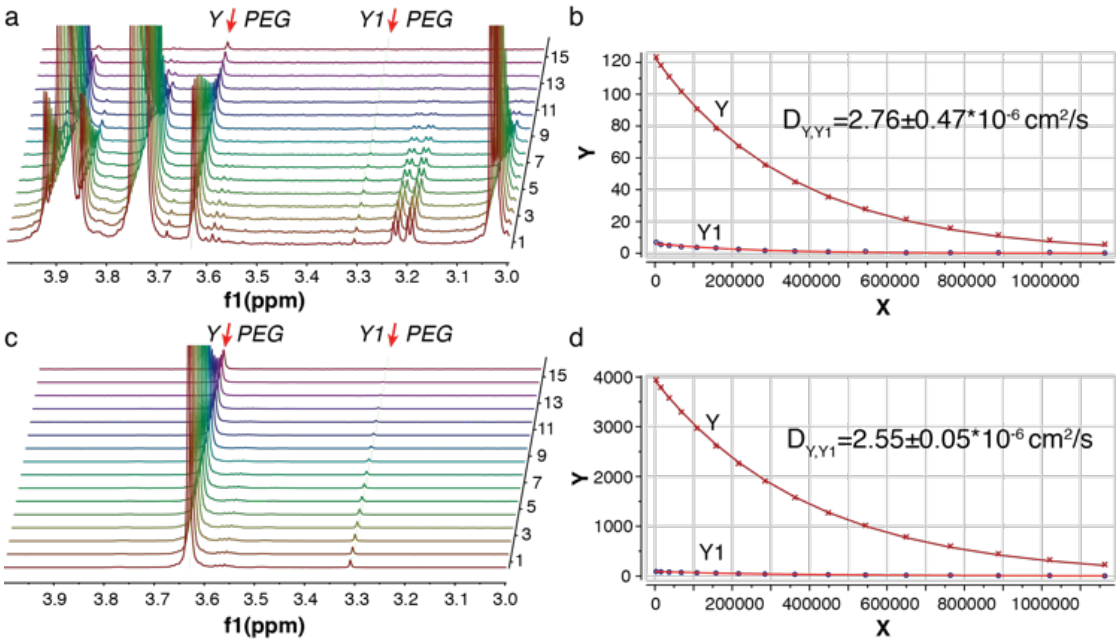
Supplementary Figure 2. a. TEM image of redox-sensitive polymersome; b. TEM image of redox-sensitive polymersome after glutathione incubation. (Scale bar: 200 nm)



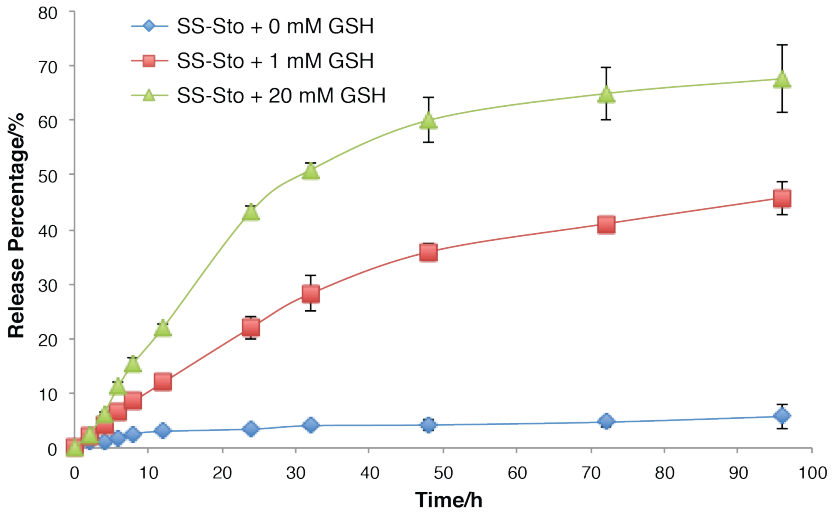
Supplementary Figure 3. DLS measurements of stomatocytes and redox-sensitive stomatocytes before and after incubating with different concentrations of glutathione. a, Size of stomatocyte before and after treating with glutathione; b, Size of redox-sensitive stomatocyte before and after treating with glutathione.



Supplementary Figure 4. Size and count rate of redox stomatocytes during the treatment with glutathione. The blue curve is the size change with time after addition of glutathione. The red curve depicts the count rate with time during the degradation.



Supplementary Figure 5. Diffusion NMR measurements of the cleaved PEG from redox-sensitive stomatocytes after glutathione treatment and free PEG in D_2O. a, Diffusion NMR spectrum of the cleaved PEG from redox-sensitive stomatocyte after glutathione treatment. b, Fitting curve of the diffusion coefficient (D) of the cleaved PEG from redox-sensitive stomatocyte after glutathione treatment. c, Diffusion NMR spectrum of free PEG (2 kDa). d, Fitting curve of the diffusion coefficient of free PEG (2 kDa). PEG signals at around 3.63 ppm and 3.31 ppm were observed from the NMR spectrum. After fitting with the equation (mono-exponential fit, B+exp(–xF)), D_Y (diffusion coefficient from PEG at 3.63 ppm) and D_{Y1} (diffusion coefficient from PEG at 3.31 ppm) were obtained. $D_{Y,Y1}$ is the average of D_Y and D_{Y1}. The diffusion coefficient of PEG cleaved by glutathione from Redox-sensitive stomatocyte was similar with that of free PEG (2 kDa), which shows the successful cleavage of PEG.



Supplementary Figure 6. The release of Dox from redox-sensitive stomatocyte nanomotors with different concentrations of glutathione.

Supplementary Table 1. Size and PDI of assembled structures

Group	Size/nm	PDI
Stomatocyte	357.1	0.069
Nanomotor	343.3	0.121
Redox Stomatocyte	349.3	0.099
Redox Nanomotor	327.4	0.110

Supplementary Table 2. Size and PDI of assembled structures before and after adding glutathione

Group	Size/nm	PDI
Stomatocyte	347.2	0.047
Stomatocyte+20mM GSH	350.0	0.065
Stomatocyte+40mM GSH	344.6	0.086
Stomatocyte+120mM GSH	350.8	0.130
Redox Stomatocyte	349.3	0.099
Redox Stomatocyte+20mM GSH	1392.0	0.700
Redox Stomatocyte+40mM GSH	2148.0	0.721
Redox Stomatocyte+120mM GSH	3095.0	1.000

Supplementary Table 3. Zeta-potential of assembled structures before and after adding glutathione

Group	Zeta/mV
Redox Stomatocyte	-30.9±0.12
Redox Stomatocyte+GSH*	-45.2±1.04

*after cleaving the PEG shell, GSH was washed away.

Reference:

(1) Yuan, W.; Zou, H.; Guo, W.; Shen, T.; Ren, J. Supramolecular micelles with dual temperature and redox responses for multi-controlled drug release. *Polym. Chem.* **2013**, *4*, 2658-2661.