Supporting Information

Temperature-Driven Switching of the Catalytic Activity of Artificial Glutathione Peroxidase by the Shape Transition between the Nanotubes and Vesicle-Like Structures

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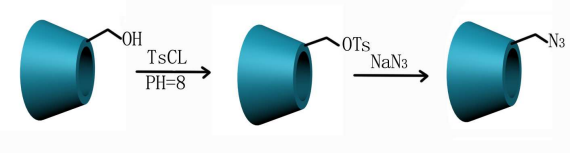
Materials

β-Cyclodextrin was purchased from Tianjinfuchen, recrystallized three times from distilled water, and dried for 12 h at 120 °C in vacuum. Tris(2-aminoethyl)amine (TREN, Acros) was used as received. Tris(2-dimethylaminoethyl)amine (Me6TREN) was synthesized as described previously.[1] *N*-Isopropylacrylamide (NIPAM) (Aldrich) was recrystallized from hexane and toluene, and dried under vacuum prior to use. Ethylenediamine, phenyl methanol and 4-toluene sulfonyl chloride were purchased from the Shanghai Reagent Co. Acryloyl chloride and propargyl alcohol were obtained from the Anhui Wotu Reagent Co. Triethylamine and tetrahydrofuran were rigorously dried with sodium.

Synthesis of azide-cyclodextrin (azide-CD)

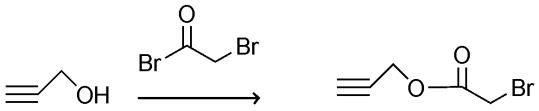
Scheme S1



6-sulfonation-β-cyclodextrin (6-TsO-CD) was prepared as described previously.[2] 6-TsO-CD (520 mg, 0.4 mmol) was dissolved in DMF (80 mL), and the sodium azide (28.6 mg , 0.44 mmol) was added into the solution. The mixture was stirred at 60 °C for 48 h. The resulting mixture was deposited with acetone for three times. The resulting solid was dried under vacuum and a white solid was obtained (417.6 mg, yield 90%). ^{1}H NMR (500 MHz, D_2O): δ 3.29-4.28 (m, 42 H), 4.76-5.19 (m, 7 H) MALDI-MS: calcd. 1159.4, found 1181.3 $[T.M. +Na]^+$.

Synthesis of prop-2-ynyl 2-bromoacetate

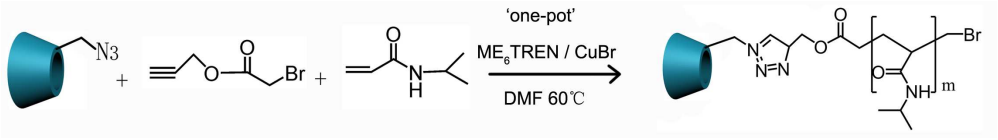
Scheme S2



Propynol (1mL, 16.9 mmol), bromoacetyl bromide (1.5 mL, 17.2 mmol), dried triethanolamine (3 mL, 21.5 mmol) were dissolved in dried dichloromethane (50 mL). The mixture was stirred for 8 hours. Removed the solvent and the product was purified by column chromatography (silica gel, dichloromethane as eluent) giving transparent liquid (2.7 g, yield 91.2%). ^{1}H NMR (500 MHz, D_2O): 4.76-4.78 (d, 2H, OCH_2), 3.87-3.88 (s, 2H, CH_2Br), 2.52-2.54 (t, 1H, C≡CH).

Synthesis of the host molecule PNIPAM-CD-Br (1c)

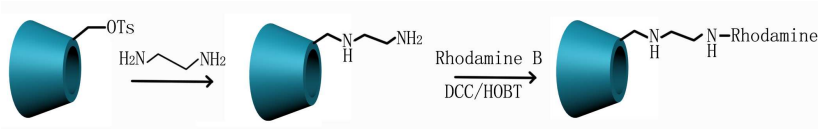
Scheme S3



PNIPAM-CD-Br was synthesized according to the polymerization procedure reported by Masci et al.[3] NIPAM (340 mg, 3 mmol, Me6TREN (11 mg, 0.049 mmol), azide-CD (305 mg, 0.026 mmol), water (3 ml) and DMF (3 ml) were introduced into a schlenk tube equipped with a magnetic bar and oil bath followed by two freeze-pump-thaw cycles. Then, CuBr (7.0 mg, 0.049 mmol) was added under nitrogen and followed by two freeze-pump-thaw cycles. Finally, the initiator prop-2-ynyl 2-bromoacetate (4.4 mg, 0.025 mmol) was added to the schlenk tube via a syringe to start the polymerization. The mixture was stirred for 48 h at 60 °C. Then the mixture was exposed to the air to terminate the polymerization and the solution was dialyzed against water for 2 days, The solid polymer was obtained via freeze-drying. NMR analysis of the polymer revealed ratio of NIPAM: cyclodextrin were 102 : 1. ^{1}H NMR (500 MHz, D_2O): δ 4.04-3.50 (m, 42 H, CD and 102 H, NIPAM), 5.12-5.02 (m, 7 H, CD), 2.20-0.80 (m, 918H, NIPAM).

Synthesis of host molecule rhodamine B- β -CD

Scheme S4



6-TsO-CD (5.2 g , 4 mmol) and ethylenediamine (70 mL) dissolved with DMF (15 mL), were stirred for 3 days at 60 °C. The resulting mixture was deposited with acetone for three times. The solid was dried in a vacuum and purified by Sephadex G-15 column chromatography with distilled water as the eluent. The solution was freeze-dried and a white solid was obtained (4.4 g, 92.9%). Then under argon, rhodamine B (240 mg, 0.5 mmol), *N,N′*-dicyclohexylcarbodie (DCC, 110 mg, 0.53 mmol) and N-hydroxybenzotriazole (HOBt, 75 mg, 0.56 mmol) were dissolved in dried DMF (10 mL) at 0 °C. The mixture was stirred for 1 h, and the ethylenediamine linkedβ-CD (500 mg, 0.42 mmol) in dried DMF (10 mL) was added to the reaction mixture, then the mixture was stirred at 0 °C for 2 hours. The mixture was stirred for 24 hours at room temperature, and then was poured into acetone (500 mL). The solid was dried in a vacuum and purified by Sephadex G-15 column chromatography with distilled water as the eluent. The resulting solution was freeze-dried and a white solid was obtained (453 mg, 65.1%). ^{1}H NMR (500 MHz, methanol-d4): δ 8.19-8.16 (m, 0.5H), 7.94-7.92 (m, 1H), 7.85-7.84 (m, 0.5H), 7.78-7.73 (m, 2H), 7.55-7.51 (m, 2H), 7.42-7.40 (m, 1H), 7.27-7.22 (dd, 2H), 7.05-7.01 (m, 3H), 6.94 (t, J = 3.0 Hz, 2H), 6.67-6.23 (m, br, 1H), 5.02-2.80(m, CDsH, overlaps with HOD and methanol). MALDI-MS: calcd. 1638 found 1602.5$[M+H-Cl]^+$.

Synthesis of cyclodextrin-based telluronic acid (1b) and adamantanol-1-3,4,5-trihydroxybenzoicamide (2)

Cyclodextrin-based telluronic acid (**1b**) and adamantanol-1-3,4,5-trihydroxybenzoic-amide (**2**) were synthesizedaccording to the previous procedures.[2,4]

Construction of the supramolecular nanotubes

The supramolecular nanotubes were constructed by direct self-assembly of CD-based host–guest superamphiphiles. The host molecule cyclodextrin-based telluronic acid **1b** (5.3 mg), **1c** (PNIPAM-CD, 27 mg), and the guest molecule adamantane-1-3,4,5-trihydroxybenzoicamide (**2,** 5 mg) were dispersed in DMF (1.2 mL) by ultrasonic till a clear solution was obtained at 30 °C. After the adamantane group was encapsulated into the cavity of CD, we carried on the second self-assembly of the fan-shaped supramolecular amphiphile in water. The amphiphile (**1b, 1c** with **2**) (100 μL) was slowly injected into a cuvette with 1.9 mL deionized water in it at 30 °C under ultrasonication. The opalescence appeared immediately, which indicated the formation of aggregates. The fine dispersion was cooled to the room temperature at ambient conditions over night for further study. We found that the stable nanotubes can be formed through self-assembly of CD-based host–guest superamphiphiles.

Characterization of self-assembled nanoenzymes by optical microscope

A drop of the sample solution was placed on a slide and sealed up using a sheet glass to keep rheumy. The process of shape transition was recorded by optical microscope through increasing the temperature from 25 °C to 45 °C and reduced back to 25 °C. The shape transition between the nanotubes and the spherical vesicle-like structures can be observed when we changed the temperature. The size distribution of the nanotube was related to the concentration. Through the test, we found that the nanotube at surfaces of the liquid droplet is bigger than the center of the liquid droplet. So the different size vesicles that from several hundred nm to 1 μm were obtained at high temperature. It is noticeable that the nanotube constructed with the host molecule PNIPAM-CD **1c**, β-CD-linked catalytic tellurium moieties **1b** and the guest molecule **2** have similar diameter with the nanotube constructed with host molecule PNIPAM-CD **1c** only and the guest molecule **2**. This means that the self-assembly behavior of the blended GPx mimic was feasible.

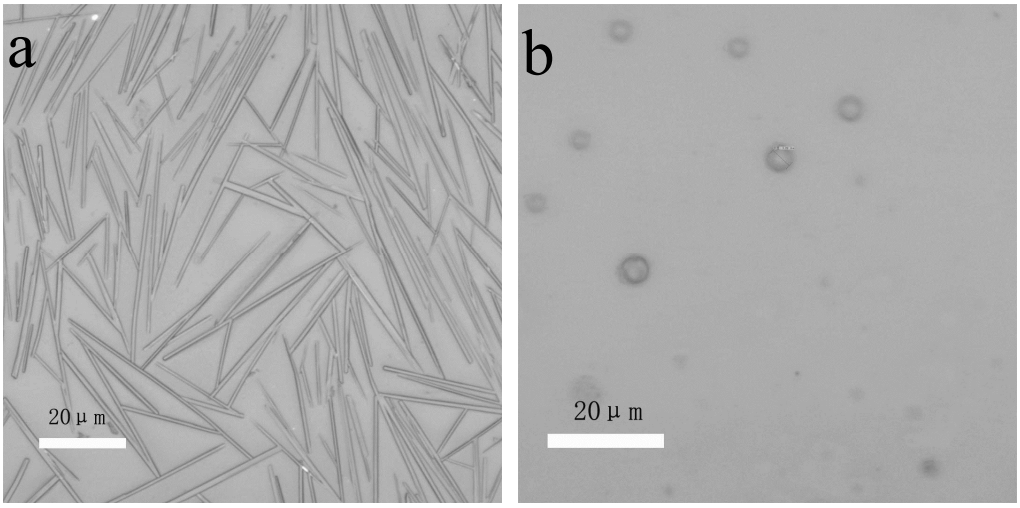
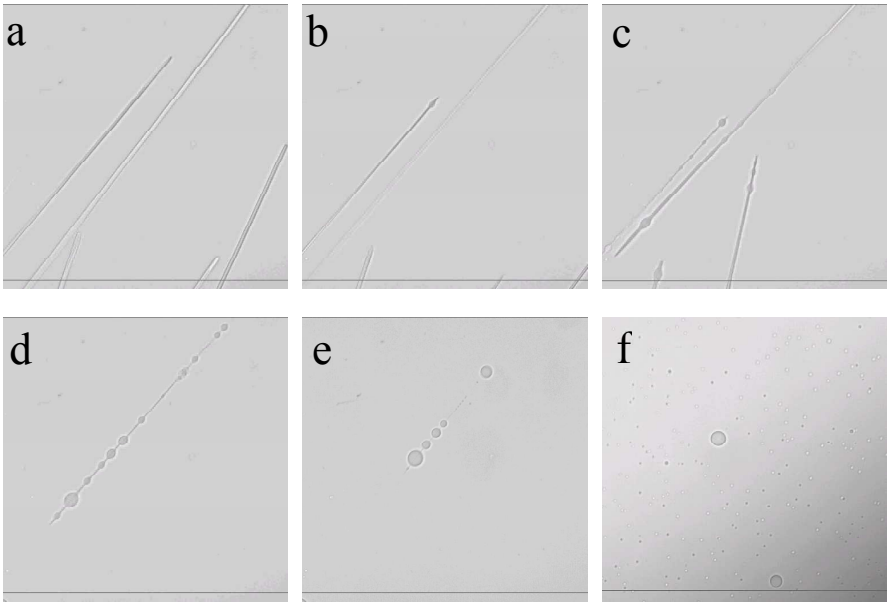


Figure S1 Optical microscope image of the nanotube and vesicle.

The reversible tube-to-vesicle transition was recorded in a video by using optical microscope. Figure S2 shows the screen shots of the reversible tube-to-vesicle transition.



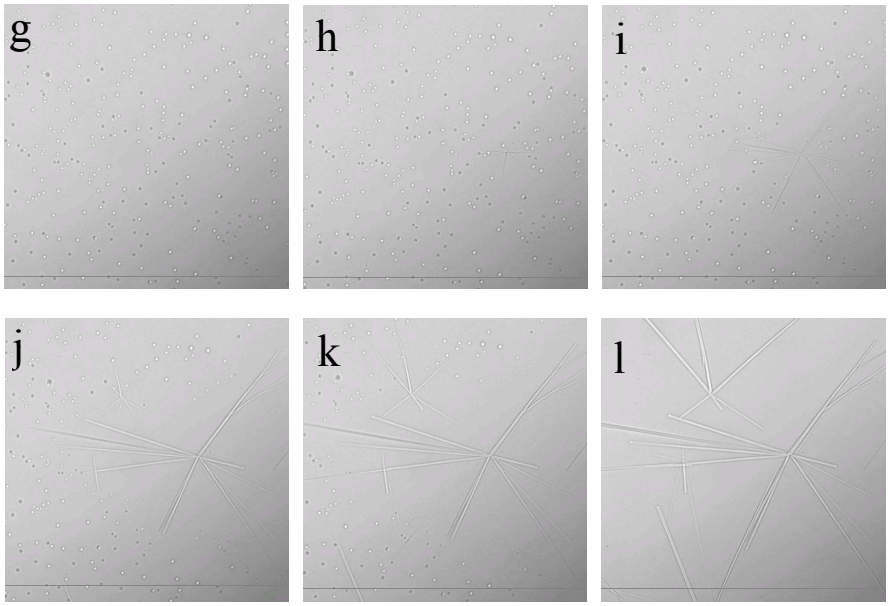


Figure S2 Optical microscope image of the reversible tube-to-vesicle transition from the video. a) the nanotube at 25 °C; b-e) the nanotubes was transferring into the vesicles when the temperature was raised from 25 °C to 45 °C; f-g) the full vesicles at 45 °C; h-k) the vesicles was transferring into the nanotubes when the temperature was decrease from 45 °C to 25 °C; l) the full nanotubes.

As a contrast, we constructed another non-thermo sensitive nanoenzyme in which the PNIPAM-CD **1c** was replaced with β-CD **1**. It maintained tubular structure even at high temperature (Figure S3). The temperature-driven switching of nanoenzyme activity could not be successfully constructed. In contrast, the mimic enzymes showed high activity at 45 °C (Figure S10).

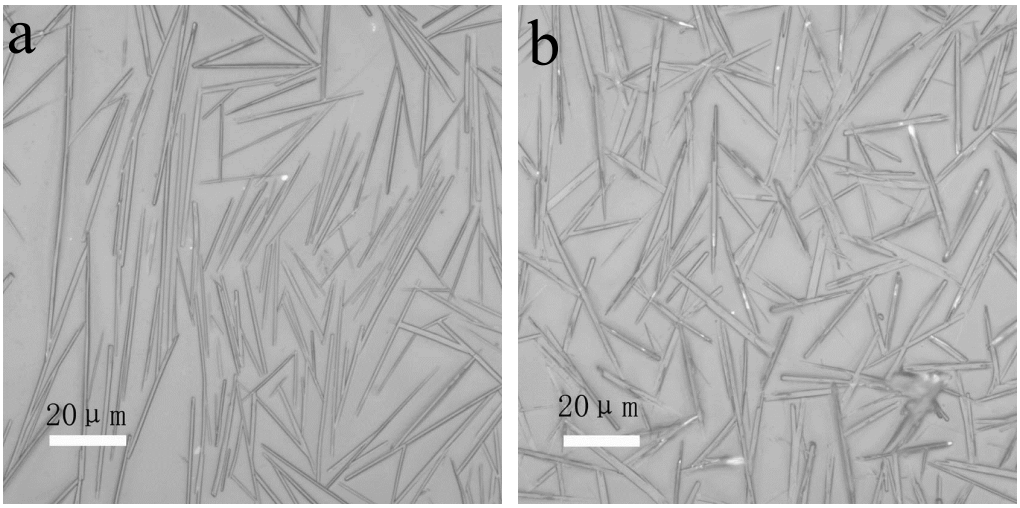


Figure S3 Optical microscope image of the nanotube. a) Constructed with host molecule β-CD 1 and the guest molecule 2 at 25 °C; b) it maintained its tubular structure even at 45 °C.

Characterization of self-assembled nanoenzymes by SEM

The morphology of the aggregates was examined by SEM observations (scanning electron microscopy, JEOL JSM 6700 F). The cryo-dried samples were prepared as following: the silicon wafers was dipped into sample at different temperature (25 °C and 45 °C) respectively, which was plunged into liquid nitrogen immediately. The water was removed from the frozen specimen by a freeze-drier. Figure S3-a shows tube-like aggregate and its diameter is about 500 nm. Figure S3-b shows the vesicle-like aggregate.

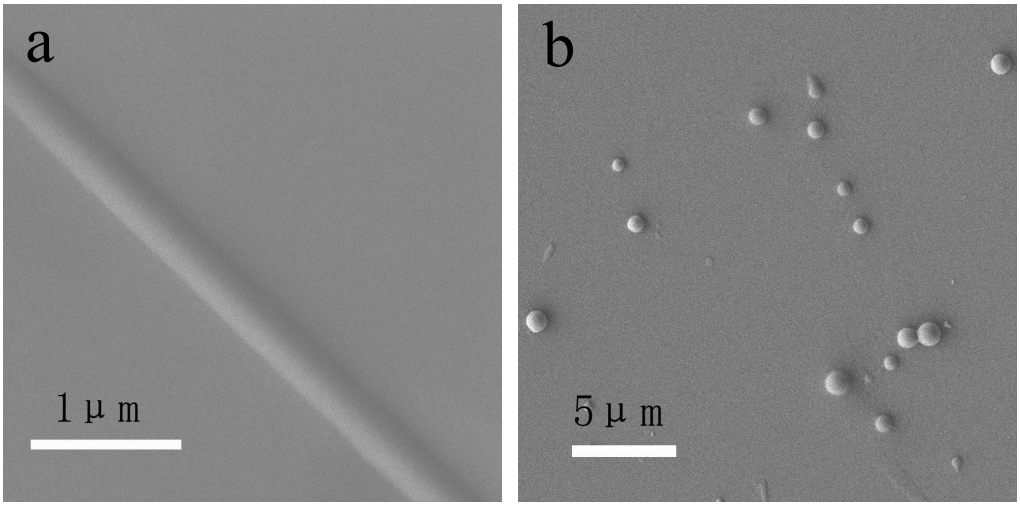


Figure S4 SEM image of the nanotube at 25 °C (a) and vesicle at 45 °C (b).

Characterization of self-assembled nanoenzymes by TEM

TEM experiments were performed with a JEOL1011 transmission electron microscope, operated at an acceleration voltage of 100 kV. The method for obtaining the cryo-dried samples is similar to SEM. Carbon-coated copper grid replaced the silicon wafers. Figure S5-a shows a strong contrast between the inner and the periphery of the tube, which is typical for tubular structures, the outer and inner diameters of these tubes are large up to 514 nm and 446 nm, respectively, and the wall thickness is about 34 nm. From Figure S4-b, we could clearly confirm the boundary between the thinner and the thicker. This observation indicated that the spherical nanoparticles formed by the GPx mimic were vesicle-like particles with a hollow structure.

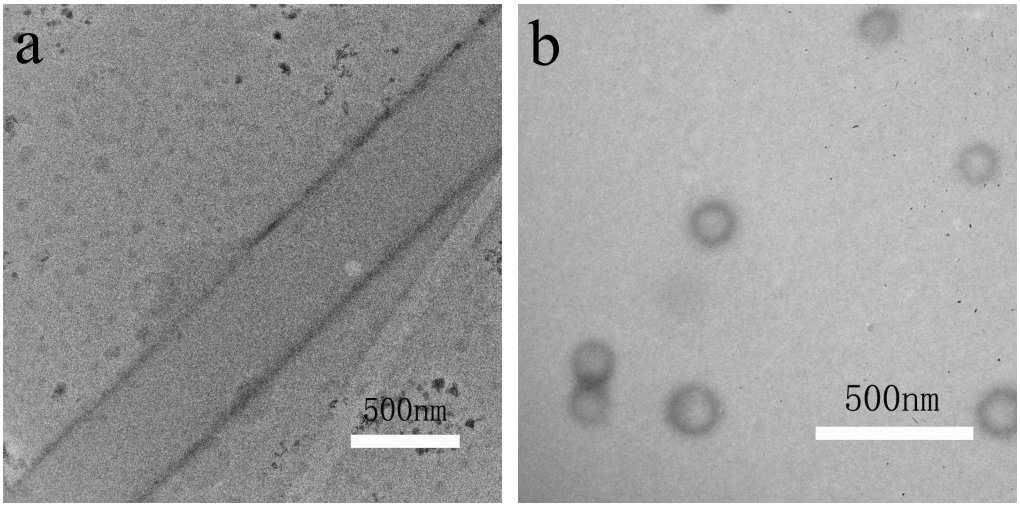


Figure S5 Transmission electron microscope image of the nanotube at 25 °C (a) and the vesicle at 45 °C (b).

Characterization of self-assembled nanoenzymes by confocal laser scanning microscope

The profile image of the nanostructures, characterized by confocal laser scanning microscopy (Olympus FLUOVIEW FV1000) was showed in Figure S6.

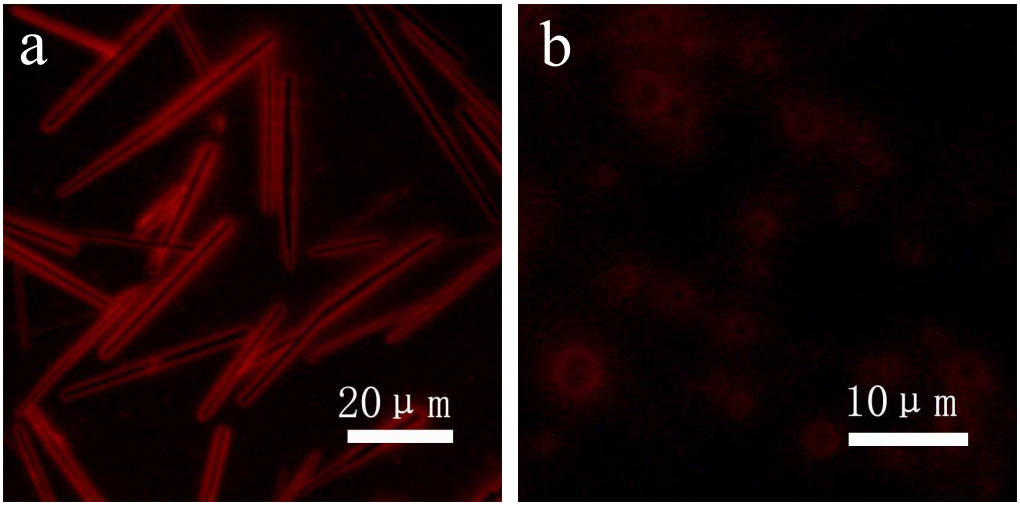


Figure S6 The confocal laser scanning microscope images of the nanotube at 25 °C and the vesicle at 45 °C.

We have done the fluorescent spectra of this system at different temperature (Figure S7), we found that the fluorescence intensity decreased at high temperature, but the peak shift remained unchanged.

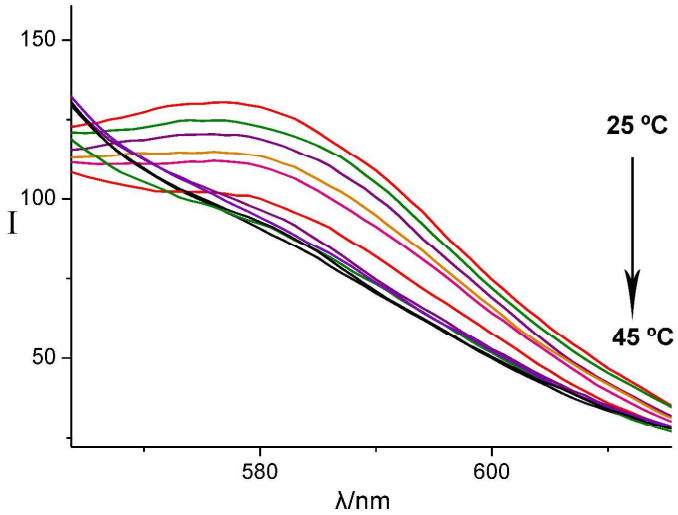


Figure S7 The fluorescent spectra of the complex from 25 °C to 45 °C. The spectra were measured at 25 °C, 27 °C, 29 °C, 31 °C, 33 °C, 35 °C, 37 °C, 39 °C, 41 °C, 43 °C, 45 °C from top to bottom.

XRD analysis

One drop of sample solution (1 mmol) was placed onto a silicon surface. The method of obtaining the cryo-dried samples was similar to SEM. The cryo-dried samples were used for XRD measurements. The Bragg peak was extracted from the XRD data and the bilayer thickness could be obtained according to the Bragg equation. Thickness of the bilayer was calculated to be 2.06 nm through three apices of XRD. Through energy optimization calculations, we deduced that the wall of the giant nanotubes is made up of about 17 bilayers of the amphiphiles.

Temperature dependence of optical transmittance of host-guest complexes (1b, 1c, 2)

The complexes (**1b**, **1c**, with **2**, 20mM dissolve in DMF) (100 μL) was slowly injected into a cuvette with 1.9 mL deionized water at 30 °C under ultrasonic. Figure S8 shows temperature dependence of the optical transmittance at 600 nm obtained in pH 7.0, 50 mM PBS.

Activity determination of nanoenzymes

The catalytic activities of these nanoenzyme models were determined by a coupled reductase method.[5] The initial rates for the reduction of H_2O_2 by GSH were determined by monitoring the decrease of NADPH absorption at 340 nm. The assay mixture contained 50 mM phosphate buffer, 1 mM GSH, 250 μM hydroperoxide

(H_2O_2) (pH 7.0), 6 μM catalyst and a moderate amount of test solution. Before investigating the activity, the catalyst was deposited in the thermostatic waterbath for 10 minutes at two different temperatures (25 ºC and 45 ºC).

First we supply the catalytic behavior of PhTeTePh and initial rates for blank at 25 ºC and 45 ºC (Figure S8).

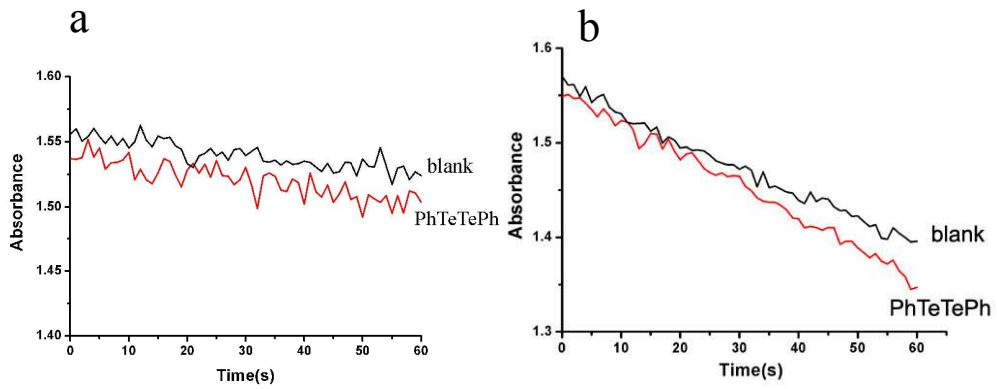


Figure S8 The GPx activities of PhTeTePh for the reduction of H_2O_2 by GSH at pH 7.0 at 25 °C (left) and 45 °C (right), respectively.

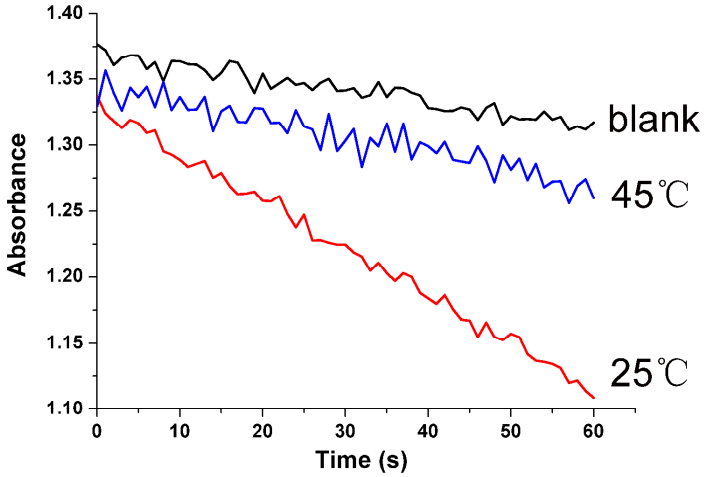


Figure S9 The GPx activities of supramolecular nanoenzymes (1b, 1c, 2) (6 μM) for the reduction of H_2O_2 by GSH at two different temperatures and pH 7.0.

In contrast, the non-thermosensitive nanoenzyme in which PNIPAM-CD **1c** was replaced with β-CD **1a**, non-thermosensitive nanotubes are incapable of morphology transition, and they maintained the tubular structures even at high temperature (Figure S5). The temperature-driven switching of nanoenzyme activity lost. In contrast, the enzyme mimics show high activity at 45 °C (Figure S10).

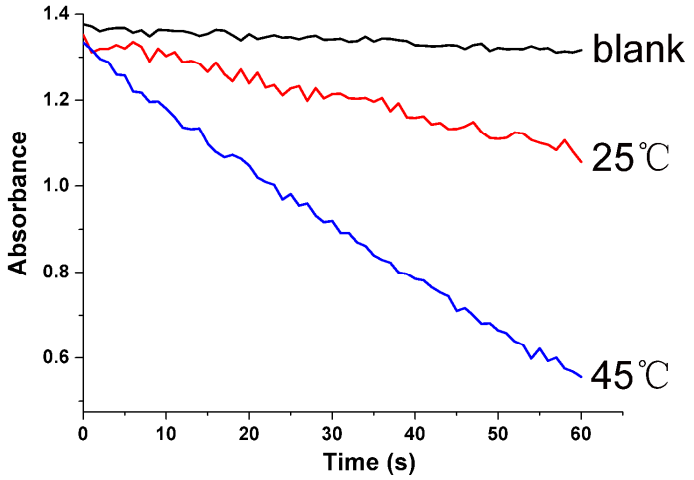


Figure S10 The GPx activities of supramolecular nanoenzymes (6 μM) for the reduction of H_2O_2 by GSH at two different temperature and pH 7.0.

The experiment in which the ratio between **1a** and **1b** is changed has been run in order to assess possible cooperativity between the catalytic centers. First the concentration of the activity center was fixed (0.5 mM), and the experiment has been done and the result indicated that the activity has not marked change when the ratio between **1a** and **1b** is changed (Figure S11).

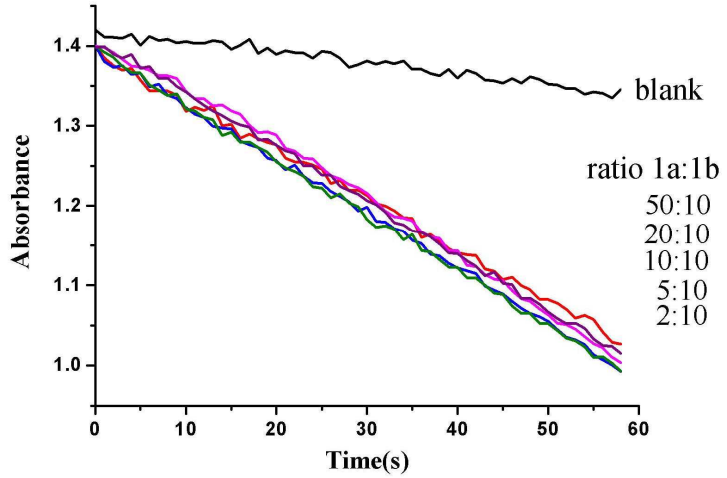


Figure S11 The activity for the reduction of H_2O_2 by GSH at the different ratio between **1a** and **1b**.

Then we fixed the concentration of the cyclodextrins, and the initial rates appeared a linear increase with increasing its concentration in our experimental condition (Figure S12).

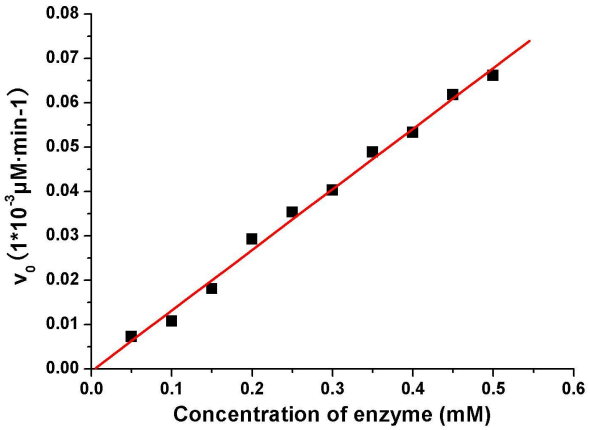


Figure S12 Plots of initial rates v_o (1×10^{-3}μM▪min-1) at different concentrations of enzyme.

For evaluating the numbers of activity sites on one vesicle, we choose a square shaped pellet area whose diameter is 2.5 μM. Ten vesicles are discovered in this area. The average radius of the vesicles is found to be 71 nm which is except the highest and the minimum value, so the average surface areas of one vesicle is about 15483 nm^2, while one β-cyclodextrin uses 2.34 nm^2 areas. We estimated the number of activity site of one vesicle was 3970 (Figure S13).

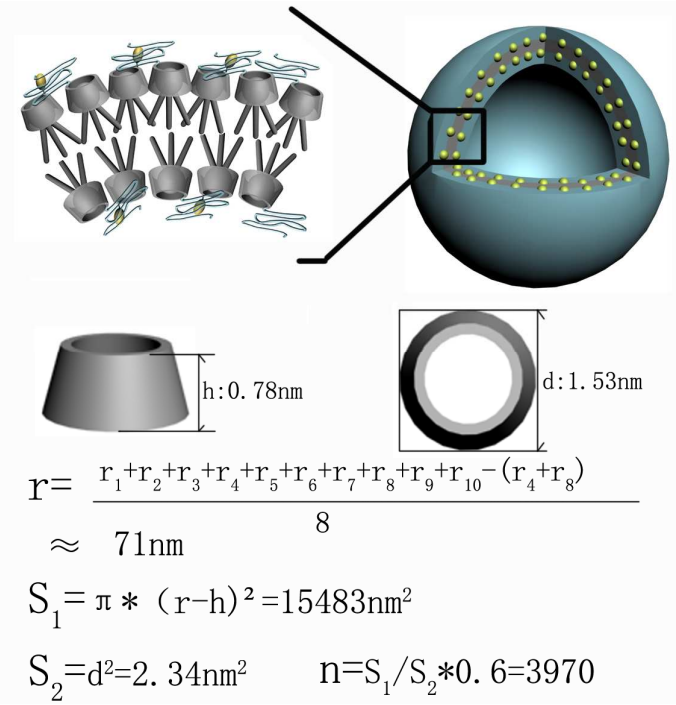


Figure S13 Evaluation of the activity sites on one vesicle. r: the average radius of the vesicles; S1: the average surface areas of one vesicle; S2: the surface of 2-β-cyclodextrin used ; n: the numbers of activity sites on one vesicle.

References for Supporting Information

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