Functionalized Nanotubes

DOI: 10.1002/anie.200907036

Giant Nanotubes Loaded with Artificial Peroxidase Centers: Self-Assembly of Supramolecular Amphiphiles as a Tool To Functionalize Nanotubes**

*Yong Tang, Lipeng Zhou, Jiaxi Li, Quan Luo, Xin Huang, Ping Wu, Yongguo Wang, Jiayun Xu, Jiacong Shen, and Junqiu Liu**

Dedicated to Professor Günter Wulff on the occasion of his 75th birthday

Molecular self-assembly, a general phenomenon in nature, plays a pivotal role in biological functions.[1] Inspired by the abundance of self-assembly processes in biological systems, considerable effort has been devoted to the design and synthesis of self-assembly-based nanostructures for their various applications in chemistry, biology, and materials science.[2,3] Among a number of other designed nanostructures, nanotubes formed by self-assembled amphiphilic molecules have attracted much attention.[4] So far various supramolecular nanotubes consisting of many identical amphiphilic molecules as building blocks have been developed.[4] The traditional types of amphiphiles (surfactants),[3–5] and also various superamphiphiles (e.g., amphiphilic block copolymers)[2f,5,6b,c] and recently giant biohybrid amphiphiles,[6] have been found to exhibit interesting self-assembly behaviors. In general, the practical methods for generating nanotubes share a common feature: the amphiphilic molecules are designed through covalent linking. Unlike these traditional covalent synthesis pathways, the "supramolecular amphiphile" concept has attracted intense scientific and technological attention.[7]

In recent years, supramolecular amphiphiles formed through noncovalent driving forces have been developed as a new type of building block for future fabrication of supramolecular architectures through multilevel self-assembly.[6,7] Among them, host–guest interactions, such as those of cyclodextrin (CD) systems, have proven to be important for constructing supramolecular amphiphiles.[7d,8,9] The self-assembly of host–guest superamphiphiles can provide opportunities not only for structural versatility but also for functional modulation of nanomaterials,[7,8] because the easy introduction of chemical moieties on host or guest molecules would provide an important tool to functionalize the self-assembled nanostructures. As examples, Zhang and co-workers reported that reversible supramolecular assemblies of an azobenzene-containing surfactant with α-CD can be reversibly controlled by photostimuli.[7b] Recently, Kim's group reported a strategy for designing nanotubes, in which the supramolecular recognition of CD and dendrons transforms the self-assembled structures from the vesicle to the CD-covered nanotube.[9] Up to now a few examples have been reported of the use of conventional CDs as host molecules to form supramolecular amphiphiles for fabricating nanostructures; however, giant nanotubes formed by directly self-assembled supramolecular amphiphiles have not yet been described.

Herein, we report a novel way to construct giant nanotubes triggered by direct self-assembly of CD-based host–guest superamphiphiles. The spontaneously formed nanotubes were functionalized with the catalytic moieties of glutathione peroxidase (GPx) by modifying the host molecule CD. Furthermore, by manipulating the surface of the nanotubes with a molecular imprinting strategy, the main catalytic components were fabricated on the scaffold of giant nanotubes. Thus, the artificial GPx center with high peroxidase activity was loaded on these well-defined nanotubes. We demonstrate that the strategy of CD-based self-assembly of supramolecular amphiphiles has great potential for the construction of functional nanomaterials and could become a powerful tool to functionalize nanotubes.

The construction of biologically functional nanotubes may open a pathway for the design of advanced materials and devices, and the fabrication of nanotubes with enzymatic function would have potential applications in biosensors and biomedicine. Thus, we wondered whether we could directly obtain nanotubes with enzymatic function by combining artificial selenoenzyme design with CD-based self-assembly of supramolecular amphiphiles in aqueous solution. This idea is very challenging, since taking a nanotube as a scaffold to construct the active sites of GPx has not yet been explored.

GPx is a well-known selenoenzyme that catalyzes the reduction of harmful hydroperoxides by glutathione (GSH), and plays an important role in the organism antioxidant defense mechanism in protecting biomembranes and other cellular components from oxidative damage.[10] In recent years, there has been increasing interest in mimicking the

[*] Y. Tang, L. P. Zhou, J. X. Li, Dr. Q. Luo, Dr. X. Huang, P. Wu, Y. G. Wang, J. Y. Xu, Prof. J. C. Shen, Prof. Dr. J. Q. Liu
State Key Laboratory of Supramolecular Structure and Materials
College of Chemistry, Jilin University
2699 Qianjin Road, Changchun 130012 (P.R. China)
Fax: (+86) 431-8519-3421
E-mail: junqiuliu@jlu.edu.cn

[**] Financial support from the Natural Science Foundation of China (No. 20874036, 20921003), the NSFC for Outstanding Young Scientists (No. 20725415), the 111 project (B06009), and the National Basic Research Program (2007CB808006) is acknowledged.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200907036.

functions of this important antioxidant enzyme, and considerable efforts have been devoted to producing organoselenium/tellurium compounds that mimic the properties of GPx.[11] To date, many artificial GPx models have been designed by introducing a selenium/tellurium catalytic center into existing or artificially generated substrate-binding scaffolds.[12]

β-CD, which has seven glucose units, can recognize the adamantyl moiety well, and this typical host–guest pair has been widely utilized to build up supramolecular materials and devices[8a–e] as it can form stable 1:1 complexes in some polar solvents, such as water and dimethylformamide (DMF).[8a–d] Thus, we selected this typical host–guest pair to prepare supramolecular amphiphiles (Figure 1). The supramolecular complex **3** can be readily prepared by the direct mixing of the guest molecule **1** and the host molecule β-CD (**2**) in the cosolvent DMF. The formation of a stable host–guest complex was driven by hydrophobic interaction between the adamantyl moiety and the hydrophobic cavity of CD.

When the supramolecular complex was dissolved in aqueous solution, compound **3** itself, as a primary building block, self-assembled into an aggregate because of its amphiphilic nature. Typically, the amphiphile (100 μL) was slowly injected into deionized water (0.9–9.9 mL) under sonication, and opalescence appeared immediately, which indicated the formation of aggregates as a result of packing-directed self-assembly. Stable assemblies could be prepared by changing the concentrations of the amphiphiles in water over quite broad ranges.[13]

Most interestingly, this supramolecular amphiphile forms unusual giant nanotubes. The optical microscopy image in Figure 2a reveals nearly monodispersed tubelike aggregates with a length of 20 μm. Scanning electron microscopy (SEM) analysis (Figure 2b) shows that a tubelike aggregate is obtained with a very large diameter (about 500 nm). The transmission electron microscopy (TEM) image in Figure 2c shows a strong contrast between the center and the periphery, which is typical for tube structures; the outer and inner diameters of these tubes are large at 510 and 430 nm, respectively,[13] and the wall thickness is about 40 nm. From calculations after energy optimization, we deduce that the wall of the giant nanotubes is made up of about ten bilayers of the amphiphiles;[13] a possible arrangement of the supramolecular amphiphile is suggested in Figure 1C. The bilayer structure of the nanotubes was further demonstrated by X-ray diffraction (XRD; Figure 3). The thickness of the bilayer was calculated to be 4.2 nm.

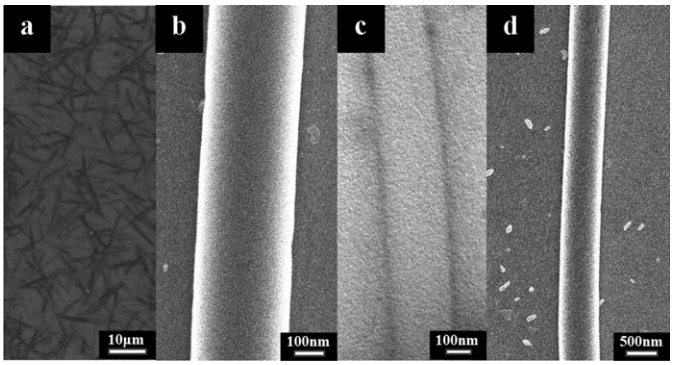

Figure 2. a, b, c) Optical microscopy (light source: white), SEM, and TEM images, respectively, of the aggregates formed by self-assembly of supramolecular amphiphile **3**. d) SEM image of the aggregate formed by self-assembly of **1** and **2b**.

The optical microscopy, SEM, and TEM images also demonstrate that the nanotubes can maintain their shapes when they are dried on solid surfaces. To further probe the structural details of the nanotube, an amino acid, L-tryptophan, with green fluorescence emission was used to modify β-CD; the self-assembly of L-tryptophan-modified β-CD (L-Trp-β-CD, **2a**) with **1** yields similarly sized nanotubes. Fluorescence microscopy revealed that the Trp moieties emitted green fluorescence in the periphery of the nanotube and demonstrated that host–guest inclusion occurred between CD and adamantanyl groups (Figure S5a in the Supporting Information). The morphology of the nanotubes was further identified by confocal laser scanning microsco-

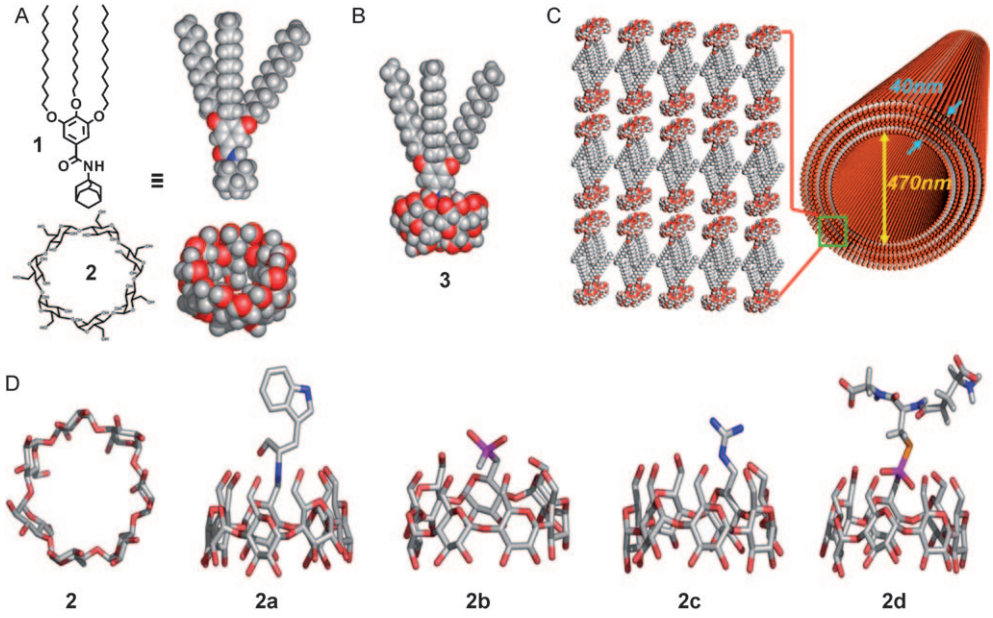

Figure 1. Structures of A) guest (**1**) and D) host molecules β-CD (**2**), L-tryptophan-modified β-CD (L-Trp-β-CD, **2a**), 6-β-CD-SeO_3H (**2bSe**) or 6-β-CD-TeO_3H (**2bTe**), 6-guanidino-β-CD (**2c**), and glutathione-modified seleno-CD (6-β-CD-Se-SG, **2d**), and B) supramolecular amphiphile **3**. C) Graphitic nanotube and possible self-assembled multilayers.

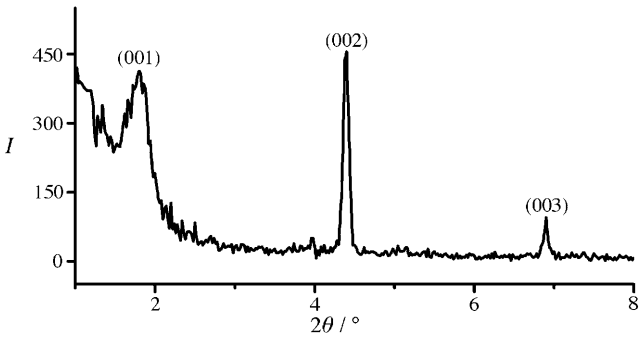

Figure 3. XRD scan of the nanotubes.

py. As shown in Figure S5b (Supporting Information), the periphery of the aggregates, except the center, has clear green fluorescence emission, which further confirms the hollow nanotube structure.

To test whether this strategy is versatile for fabricating nanotubes with enzymatic function, various host molecules with functional groups related to GPx catalysis, containing β-CD-linked catalytic selenium or tellurium moieties (**2b**) or guanidine-functionalized β-CD (**2c;** Figure 1), were designed by introducing them into the C6 position of β-CD.[13] After the host molecules were changed to these functionally modified CDs or a combination thereof, the same self-assembly procedures were carried out for these supramolecular amphiphiles. Nanotubes with similar morphology and size to those mentioned above were obtained (Figure 2d and Figure S5 in the Supporting Information). Thus, we have demonstrated that this strategy is a feasible way to fabricate the active site of GPx on the nanotube surface.

In the active site of GPx,[10] a catalytic moiety (selenocysteine) is located in a specific GSH binding site, and the substrate GSH is stabilized by two arginine (Arg57, Arg103) and other residues (Figure 4a). Being similar to the native GPx, CD-based selenonic acid (6-CD-SeO_3H, **2bSe**) or telluronic acid (6-CD-TeO_3H, **2bTe**) were designed to imitate the selenocysteine function, and were used in the self-assembly process (Figure 4).

The catalytic activities of the nanotubes were first evaluated in an assay system using 3-carboxy-4-nitrobenzenethiol (TNB) as a GSH alternative.[13,14] Obvious rate enhancement for the reduction of cumene hydroperoxide was observed in the case of selenium-containing functional nanotubes.[13] The nanotube catalyst with a tellurium center showed an increasing velocity compared to the nanotube with a selenium center. For enhancing the capacity of substrate binding, we introduced guanidine groups on the nanotubes as recognition sites by mixing guanidine-functionalized β-CD (6-guanidino-CD, **2c**) with CD-TeO_3H (**2bTe**) in a 1:1 molar ratio, which is similar to the recognition function of arginine residues in the GPx catalytic center (Figure 4a). Thus, the nanotube was endowed with both an active site and a recognition site, and their combination further increased the catalytic capacity.[13] Typical saturation kinetics of nanotube catalysis proved that the nanotubes loaded with selenoenzyme catalytic centers behaved as real catalysts.[13]

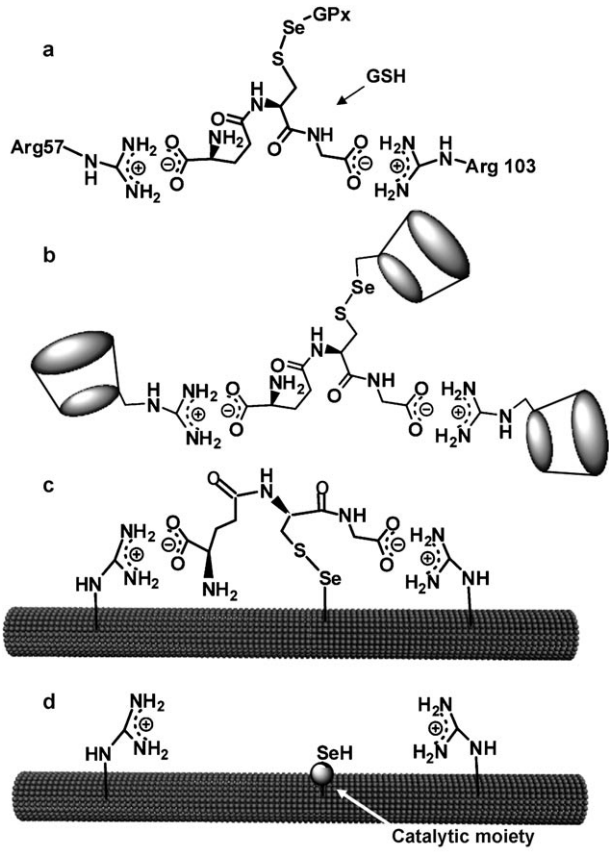

Figure 4. Preparation of the GPx catalytic center on nanotubes by the combination of molecular self-assembly and an imprinting strategy. a) GPx active site with a GSH binding site;[10] b) complex of the substrate GSH and guanidino-CD; c) fixing the conformation of the complex on nanotubes by a self-assembly process; and d) the designed active center of GPx with a specific binding site for GSH and oriented catalytic site.

In GPx catalysis, the tripeptide GSH acts as a natural substrate;[10] when we used GSH as substrate, the catalytic activities of the nanotubes were also evaluated in a GSH reductase–reduced nicotinamide adenine dinucleotide phosphate (NADPH) coupled assay system.[12d,f] The nanotube with a selenium center (NT-CD-SeO_3H, **2bSe**) showed a GPx activity of 3.6 U for the reduction of H_2O_2 by GSH (Table 1), and the introduction of guanidine groups as recognition sites

Table 1: GPx activities of supramolecular nanotubes for the reduction of H_2O_2 by GSH at pH 7.0 and 37 °C.[a]

Catalyst	Substrate	Specific activity [U]
ebselen[11c]	GSH	0.99
NT (**1**, **2bSe**)[b]	GSH	3.6
NT (**1**, **2bSe**, **2c**)[b]	GSH	54
IM-NT (**1**, **2d**, **2c**)[b]	GSH	140

[a] The initial rate of reaction was corrected for spontaneous oxidation in the absence of catalyst in phosphate-buffered saline (pH 7.0) at 37 °C. One molecular catalytic center (selenium moiety) was defined as one active site. All values are means of at least three repeats with an error of less than 5%. [b] NT: nanotube.

on the nanotubes increased the GPx activity to 54 U. In a catalytic cycle, substrate binding and the orientation of catalytic groups are responsible for efficient catalysis.[15,16a,17] To generate a specific GSH binding site and to incorporate and orient the catalytic moiety selenium into the binding site, a molecular imprinting strategy was used to manipulate the surface of the nanotubes. Molecular imprinting is regarded as a promising strategy for generating selective sites, and has been widely used for constructing artificial enzymes.[16]

As shown in Figure 4, the covalent conjugate of GSH and seleno-CD (CD-Se-SG, **2d**)[12c,13] was used as a template to create a complementary binding site of GSH on a nanotube. Recent studies have shown that the guanidinio group can complex with carboxylate groups through stoichiometric noncovalent interaction.[17] After pre-organization of the GSH unit of **2d** with the guanidine moieties of two **2c** molecules (Figure 4b), the conformation of the complex of GSH and 6-guanidino-CD was fixed on the surface of the nanotubes by a similar self-assembly process to that mentioned above. Thus, the catalytic selenium group is successfully oriented in the GSH binding site in proximity to the reacting sulfhydryl moiety of GSH in the active site (Figure 4c,d), which is similar to the active site of natural GPx (Figure 4a).[10] This conformation might facilitate intramolecular catalysis. Experiments revealed that the imprinted nanotubes showed a further increased activity of 140 U (Table 1), which is approximately 2.6 times that for unimprinted nanotubes. Compared with ebselen,[11] a well-known GPx mimic, a remarkable enhancement of 140-fold was observed.

In conclusion, we have developed a novel strategy for the facile preparation of giant nanotubes based on self-assembly-driven host–guest interaction between CD and guest molecules, with the resulting superamphiphiles further self-assembled into nanotubes. This approach is simple, economical, and environmentally benign and might be applicable to the synthesis of other functional nanomaterials. Meanwhile, the nanotubes were functionalized through carrying selenium or tellurium as the catalytic center of GPx, which made the nanotubes an efficient peroxidase scaffold. The activity of nanotube catalysts was enhanced, to some extent, when specific substrate binding sites were introduced by a molecular imprinting strategy. As a new kind of nanomaterial, enzyme-based nanotubes demonstrate potential applications in biosensors and biomedicine. This strategy can be used as a platform for building more-complex biologically functional nanomaterials and devices.

Received: December 14, 2009
Published online: April 29, 2010

Keywords: amphiphiles · cyclodextrins · host–guest systems · nanotubes · self-assembly

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