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Reversible vesicles of supramolecular hybrid nanoparticles†

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Inorganic nanoparticles can self-assemble into various supra-structures, among which vesicles are seldom reported. In this paper, a reversible self-assembly of gold nanoparticles to vesicles, mediated by double hydrophilic block copolymers, was carried out in pure water under the stimulus of temperature.

Inorganic nanoparticles (NPs) are of great importance as building blocks in constructing various supra-structures using the toolbox provided by supramolecular chemistry. The possible collective properties of such supra-structures are associated to the size, shape, and detailed organization of the assemblies, which motivate the current research.[1–4] Thus the self-assembly of NPs becomes more and more fascinating to chemists and material scientists. Great successes have been made in fabricating various delicate supra-structures, including ribbons,[5] strings,[6] dendrite structure,[7] rings,[8] double helices,[9] *etc.*, from inorganic NPs mediated by all kinds of organic building blocks, including peptides,[10] oligonucleotides,[11] small molecules,[12] surfactants,[13] and polymers.[14–21]

As one kind of effective organic mediator for the self-assembly of NPs, block copolymers can form various assembled structures, including micelles, vesicles, worm-like micelles *etc*,[22,23] which provide plenty of room in controlling and adjusting the self-assembly of NPs. Among the various resultant supra-structures of NPs formed with the aid of block copolymers and other organic mediators, vesicles[24] might be the most noteworthy because of their closed hollow sphere structure with a rather thin wall, which can be used as functional nanocarriers. Furthermore, vesicles, as a prototype of living cells, are very important in helping to understand the nature. Until now, there were only few brilliant cases of constructing a vesicular wall from NPs,[13] under the help of block copolymers,[16,17] or even homopolymers.[19] However, in the research of such NP-based vesicles, the disassembly process has been ignored. In other words, the reversibility of such processes involving stimuli-responsive NPs has not been reported.

Aiming at reversibility of the hybrid self-assembled structures, the concurrent self-assembly of an inorganic species with block copolymers is preferred. In the previously reported cases, formation of the

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hybrid vesicle structure was realized in mixed media containing organic solvent, which of course is not practical to pursue full reversibility.[16,17] In this paper, a stimuli-responsive block copolymer was tailor-made and introduced to prepare hybrid vesicles with gold nanoparticles (AuNPs) by supramolecular means. Hybridization of AuNPs with the copolymers as well as the subsequent self-assembly into vesicles in water can be reversibly controlled. Furthermore, the vesicle wall formed here consists of a monolayer of AuNPs, which is rarely reported in literature and makes the corresponding mechanism of vesicle formation clear.

Recently, we reported a supramolecular structure named HIC (hybrid inclusion complex), in which quantum dots were non-covalently modified by block copolymers *via* inclusion complexation.[25] In this communication, a new HIC with an AuNP core covered with α-cyclodextrins (Fig. 1) and supramolecularly attached block copolymers serves as a building block to form thermally induced reversible hybrid vesicles. The attachment of the block copolymers to the NP surface was realized by a photo responsive host–guest pair, *i.e.* α-cyclodextrins (α-CDs) and Azobenzene (Azo), the latter was the end group of the block copolymer poly(*N*-isopropyl acrylamide-*b*-*N*,*N*-dimethyl acrylamide) (Azo-PNIPAM-*b*-PDMA). The well-known thermal responsive "coil-to-globule" transition of the PNIPAM block was crucial for the reversible formation of hybrid vesicle structure. Different from the previous methods in organic solvents, formation of hybrid vesicles and its subsequent dissociation in current research can be achieved in water. Such an organic-solvent-free method provides new opportunities for further development of hybrid materials potentially useful in bio-related areas.

AuNPs (α-CDs@AuNPs) were prepared using perthiolated α-CDs as protective groups[26] and characterized by ^{1}H NMR in D$_2$O. The peaks belonging to α-CDs were broadened due to their more restricted mobility on the particle surface compared to that of free α-CDs (Figure S1, ESI†). The diameter of the AuNPs was found to
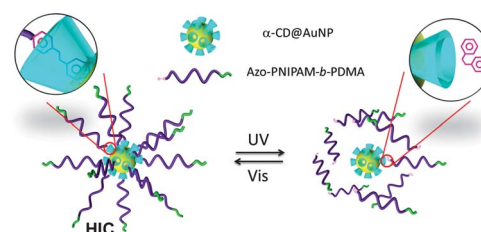

Fig. 1 Photo reversible formation of HIC by α-CD@AuNP and block copolymer Azo-PNIPAM-*b*-PDMA.

be about 3 nm by transmission electron microscopy (TEM), as shown in Figure S2a.† A relatively weak plasmon resonance absorption with a maximum at 497 nm was observed by UV-vis spectrum (Figure S2c†), which was a feature property of AuNPs within this size range. Furthermore, the residual weight fraction determined by thermogravimetry analysis (TGA, Figure S2d†) was 80%, which meant that the weight content of α-CDs was 20%. So the average number of α-CDs on each AuNP could be estimated as 28.[27] Size distributions of the AuNPs (0.1 mg mL^{-1} in water) determined by dynamic light scattering (DLS) are shown in Figure S3,† which indicated that over a broad temperature range, these α-CD@AuNPs were well-dispersed in water without aggregation. Block copolymer Azo-PNIPAM-b-PDMA was synthesized via reversible addition fragmentation chain transfer (RAFT) polymerization using the chain transfer agent (Azo-CTA) reported by our group[25] and characterized by GPC and ^{1}H NMR (supporting information, Fig.S4–S5, ESI†). The degrees of polymerization of NIPAM and DMA determined by ^{1}H NMR were about 168 and 39 respectively. The surface modification of α-CD@AuNPs by Azo-PNIPAM-b-PDMA was driven by inclusion complexation between α-CD and Azo when they were mixed in aqueous solution, forming the structure of HIC[25] (Fig. 1). After an excess of Azo-PNIPAM-b-PDMA was mixed with AuNPs, the aqueous solution was dialyzed against deionized water (50 kDa molecular weight cut off). TGA analysis of the sample after freeze-drying demonstrated that the organic content of HIC was around 88.7% (Fig. S6†), which was very close to the calculated value, assuming the CD cavities were fully occupied by the Azo moieties (see ESI†). This result shows the successful fabrication of the hybrid structure (HIC). For the following studies in this work, the concentration of HIC in aqueous solution was kept at 0.1 mg mL^{-1}, unless specially mentioned.

It is known that the Azo moiety performs trans-to-cis isomerization under irradiation of UV light, while a reverse process takes place under visible light. Azo moieties in the HIC structure retain this photo-responsive property, as shown in Fig. 2a. After UV irradiation, the absorption band near 300 nm corresponding to trans-Azo disappeared, and appeared again after subsequent irradiation by visible light ($\lambda = 420$ nm). This property is a key fact in supramolecular chemistry as trans-Azo binds to α-CD, while its cis isomer does not. Thus the formation and dissociation of HIC can be controlled by light irradiation. As shown in Fig. 2b, the reversible attachment and de-attachment of the block copolymers on the AuNP surface can be visualized by dynamic light scattering (DLS). The hydrodynamic
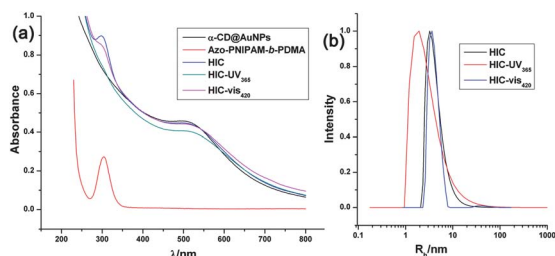
radius (R_h) of HIC is around 4 nm, compared to the diameter (ca. 3 nm) of AuNP itself found under TEM. The increase of size indicated the successful non-covalent modification of α-CD@AuNPs with block copolymers. After UV irradiation, R_h was found to be around 2 nm, showing the "naked" α-CD@AuNP, which returned to 4 nm with a similar size distribution to that of HIC, after subsequent visible light irradiation.

It was reported that, AuNPs covered by homopolymer PNIPAM easily precipitated from random aggregation at elevated temperature.[20] In our case, although HIC inherits the thermo-sensitive property of PNIPAM, above its lower critical solution temperature (LCST) around 32 °C, no precipitate was observed due to the presence of the short hydrophilic block of PDMA at the outer layer of HIC. However, when the solution was heated to around 32 °C, larger, narrowly-distributed and stable aggregates formed as the hydrodynamic radius $<R_h>$ drastically increased from 4 nm to 110 nm, accompanying an apparent decrease of the polydispersity index (PDI) from about 0.5 to 0.1 (Fig. 3a). When the temperature was increased to 36 °C, $<R_h>$ finally reached 160 nm without much change at even higher temperatures, while a remarkable Tyndall effect can be observed (Fig. S7a, ESI†), indicating the formation of stable and well-organized structures. Surprisingly, as shown in Fig. 3b, when several heating-cooling processes were performed between 24 °C and 32 °C, the changes of $<R_h>$ and PDI were completely reversible and reproducible within 30 min, indicating robust reversibility for the construction of the large assemblies observed at elevated temperature. As a control experiment, when PNIPAM homopolymer capped by Azo groups to the same degree of polymerization (Azo-PNIPAM) was employed to form HIC (HIC-homo), without the hydrophilic PDMA block, microscopic precipitation from the aggregation of HIC-homo was easily observed at 32 °C, as shown in Figure S7, ESI.† It is reasonable that due to the absence of hydrophilic protection, when PNIPAM became hydrophobic above LCST, the HIC aggregated randomly in the large scale to form microscopic precipitation.

This reversible transition between single hybrid particles and large assemblies explored by DLS resembles the results reported by us[28] and other research groups,[29–32] i.e. block copolymers with PNIPAM as the main and middle block with one hydrophobic and one hydrophilic side component performed a micelle-to-vesicle transition while the solution in water was heated above the LCST. The obtained vesicles exhibited much larger diameters and narrower PDI than micelles, which was similar to the result of this work. To further explore the morphology of the assembled HIC sample at 32 °C, TEM was performed (Fig. 4a–c). The radius of globular structures in
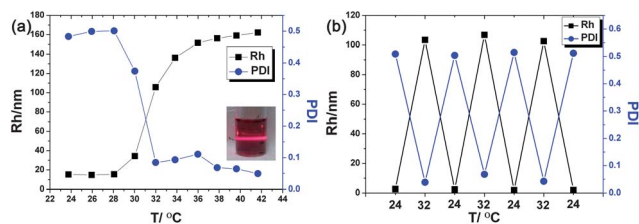

Fig. 2 (a) UV-vis absorption spectra of α-CD@AuNP (0.1 mg mL^{-1}, black), Azo-PNIPAM-b-PDMA (0.5 mg mL^{-1}, red), HIC (blue), HIC after UV irradiation (HIC-UV$_{365}$, $\lambda = 365$nm, green), and after subsequent visible light irradiation (HIC-UV$_{420}$, $\lambda = 420$nm, pink) in aqueous solution at 25 °C. (b) The corresponding DLS result of HIC after subsequent UV and visible light irradiation.

Fig. 3 (a) DLS study of HIC in aqueous solution with $<R_h>$ and PDI responding to increasing temperature, the inserted photo was taken at 42 °C; (b) Reversible changes in R_h and PDI with temperature cycles between 24 °C and 32 °C.
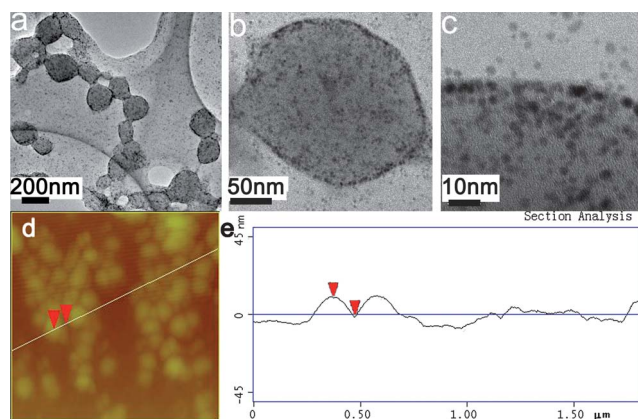
Fig. 4 TEM images (a,b,c) and AFM analysis ((d) top-view, (e) section analysis of height) of HIC-vesicles prepared at 32 °C.

Fig. 5 (top) Structure of Azo-PNIPAM-*b*-PDMA. (bottom) ¹H NMR study of HIC in D₂O (10 mg mL⁻¹) at varied temperature.

Fig. 4a is about 100 nm, which is in accordance with the DLS results. The image of one globular structure at a higher magnification in Fig. 4b presents the hollow feature of vesicles, as the periphery looks much darker than the inner portion.

The thickness of the membrane of the vesicles is estimated to be around 5 nm, as shown in Fig. 4c. Moreover, it is obvious that the membrane wall contains a monolayer of AuNPs. AFM was also used to characterize the hollow feature. As shown in Fig. 4d–e, the height of these collapsed vesicles on a mica surface is only about 10 nm, which is in accordance with two overlapped layers of the hybrid nanoparticles. In the literature,[33–35] such soft vesicles made by block copolymers that had collapsed on the surface also exhibited images with a very low height obtained by AFM compared to their diameter. During the drying process, the vesicles usually collapse as a result of solvent evaporation from their inner cavity. Hence, the height of the collapsed vesicles on the surface normally corresponds to double the membrane thickness of the vesicle in solution.[36] Thus we conclude that the spheres of HIC assemblies we observed upon LCST by DLS are vesicles (named HIC-vesicles). Moreover, the hollow sphere has a monolayer of AuNPs in their thin wall, which is an unusual feature in vesicles of AuNPs compared with those prepared from organic solvent.[17]

¹H NMR study gave further insight into the self-assembly process on the molecular level. As shown in Fig. 5, when HIC was heated at 32 °C, the intensity of the signals assigned to the PNIPAM block (chemical shift δ around 3.7) decreased, while those of the PDMA block changed little, indicating that the PNIPAM block became hydrophobic and collapsed on the nanoparticle surface, while the PDMA block stayed solvated and separated from the collapsed PNIPAM phase (Scheme 1b). Driven by minimizing the surface energy between the collapsed PNIPAM chains and water, the collapsed HIC assemblies as building blocks tend to come into contact with each other and then fusion between the connected parts takes place. Fusion reduces the surface areas of the collapsed HIC, which also makes the density of hydrophilic moieties close to the fusion area increase. The densified hydrophilic moieties ("close" part in Scheme 1c) retards further fusion close to this area and, as a result, it directs the next combination step of the HIC through the "open" part (Scheme 1c), then finally leads to two dimensional patches. In fact, in the literature that soft species such as lipids,[37] reactive cucurbit[6]uril[38] and polymeric micelles[39] connect to each other laterally rather
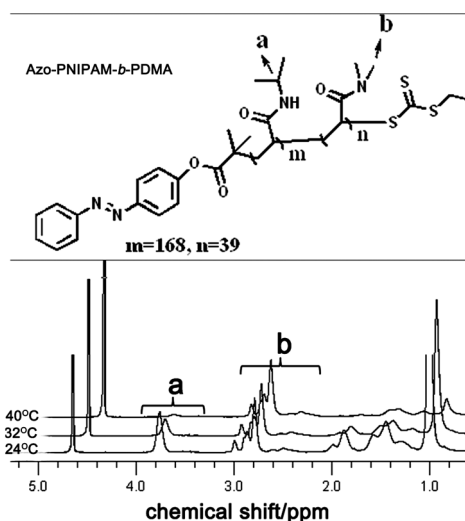
than randomly has been commonly observed and theoretically proved. Finally the assembled patch starts to bend, forming vesicles driven by reducing the total energy. Although this "collapse–fusion–bending" process cannot be traced by TEM observations, the monolayer structure of AuNPs on the vesicular wall observed does support this hypothesis of the mechanism. It is worth mentioning that, for the nanospheres as hard as AuNPs, coating with a dense layer of block copolymers renders the particles with enough softness to make their further self-assembly follow the way of soft particles.[39] Moreover, because the layer of block polymers on the surface of the hard spheres is dense, in the resultant vesicular wall the NPs are immersed into the collapsed PNIPAM matrix and thus, under UV light irradiation at 32 °C, no dissociation of the HIC-vesicles was observed although the non-covalent connection of the copolymers to AuNPs was dissociated.

In short, mediated by double hydrophilic block copolymer, the formation of vesicles from AuNPs has been successfully performed in pure water, with a satisfactory reversibility and rapid transition speed that cycles in minutes. Although the thermo-responsiveness of PNIPAM itself is well known, it is the first demonstration that this property results in the reversible formation and dissociation of hybrid vesicles of AuNPs, as far as we know. Furthermore, the vesicle wall is as thin as a single layer of AuNPs, which should be highlighted as an interesting feature of this structure. For biological applications of the
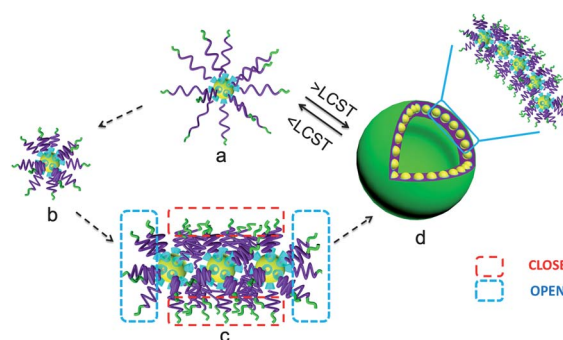
Scheme 1 A possible mechanism of HIC-vesicle formation.

vesicles in the near future, the trithiocarbonate end of the copolymer can be converted to a thiol group[40] in order to incorporate more functionalities on the vesicle surface and the transition temperature to form vesicles can be increased to 37 °C by copolymerizing a hydrophilic monomer into the PNIPAM chain.[41]

Acknowledgements

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