VIP Polymer Nanocapsules

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Direct Synthesis of Polymer Nanocapsules with a Noncovalently Tailorable Surface**

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Nanometer-sized hollow polymer spheres or polymer nanocapsules are important for a wide range of applications including drug delivery, encapsulation, and imaging.[1] Several methods to synthesize such polymer nanocapsules have been reported, such as template synthesis,[2] self-assembly,[3] emulsion polymerization,[4] and core removal of dendrimers.[5] Although each of these has its own merits, they all need either a preorganized structure or template to shape a hollow shell structure, and furthermore they require time-consuming and laborious multistep processes including removal of the core or templates, repeated centrifugation or filtration, cross-linking of specially designed vesicular species, or separation of large quantities of surfactants. In addition, the facile modification of the nanocapsule surface is important for various applications such as targeted drug delivery, but tailoring the surface properties has not been an easy task. Here, we describe the direct synthesis of polymer nanocapsules by polymerization of a rigid, disk-shaped host molecule with a cavity and multiple polymerizable groups at the periphery. This method, which appears to be applicable to any monomer with a flat core and multiple polymerizable groups at the periphery, directly produces polymer nanocapsules with a highly stable structure and relatively narrow size distribution without the need for any preorganized structure, emulsifier, or template.[6] Another feature of this method is that the polymer shell, which comprises a host molecule, allows facile tailoring of its surface properties in a noncovalent and modular manner by virtue of the unique recognition properties of the accessible molecular cavities exposed on the surface.

Polymer nanocapsules **3** were directly synthesized by the thiol-ene photopolymerization[7] of $(allyloxy)_{12}$cucurbit[6]uril (**1**),[8–9] a rigid disk-shaped molecule with a cavity and 12 polymerizable allyl groups at the periphery, and dithiol **2** (Figure 1). In a typical experiment, UV irradiation of a mixture of **1** and **2a** in 1:48 ratio (allyl/thiol = 1:8) in methanol

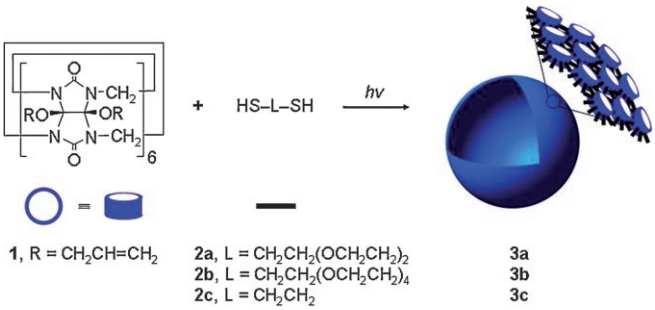

Figure 1. Synthesis of polymer nanocapsules **3**. Disulfide loops protruding from the surface of polymer nanocapsules are omitted for clarity (see Figure S3 in the Supporting Information).

for 20 h followed by dialysis produced polymer nanocapsule **3a** in 87 % yield based on **1**. The product was characterized by various spectroscopic and imaging techniques. The FTIR spectrum of **3a** revealed two characteristic peaks of the cucurbit[6]uril (CB[6]) unit (C=O and C–N stretching vibrations), but no peaks corresponding to the allyl and thiol groups of the starting materials (see Figure S1 in the Supporting Information). Solid-state NMR spectroscopy also confirmed the formation of new thioether bridges and the disappearance of the allyl groups (see Figure S2 in the Supporting Information). Elemental analysis showed that the ratio of **1** and **2a** incorporated into **3a** is 1:15.5 but decreases to 1:7.4 after treating **3a** with excess ethyl vinyl ether under UV light. This result suggests that upon reaction of **1** with **2a**, approximately nine of the twelve allyl groups of **1**

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form thioether bridges that link neighboring CB[6] units to yield a two-dimensional (2D) polymer network that constitutes the nanocapsule shell. Meanwhile, the remaining allyl groups form disulfide loops that protrude from the nanocapsule surface and can be removed by the treatment with ethyl vinyl ether (see Figure S3 in the Supporting Information).

Dynamic light scattering (DLS) studies revealed that the polymer nanocapsule **3a** has an average diameter of (110 ± 30) nm, which is in good agreement with the size observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM) studies (Figure 2a, b). Furthermore, a combination of dynamic and static light scattering studies

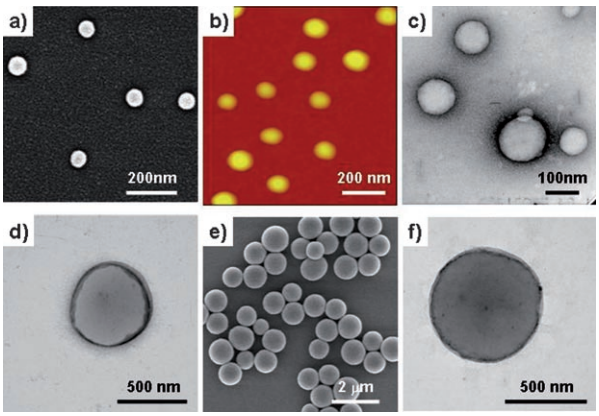

Figure 2. Microscopy images of polymer nanocapsules. a) SEM, b) AFM, and c) TEM images of **3a** prepared in methanol. d) TEM image of **3a** prepared in chloroform. e) SEM and f) TEM images of **5a** prepared in acetonitrile.

showed that the radius of gyration ($R_g = 60.2$ nm) and the hydrodynamic radius ($R_h = 57.3$ nm) are almost the same ($\rho(R_g/R_h) = 1.05$), indicating the hollow nature of **3a**.[10] High-resolution and cryo transmission electron microscopy (TEM) studies revealed a hollow interior surrounded by a thin shell with an average thickness of (2.1 ± 0.3) nm (Figure 2c as well as Figure S4 in the Supporting Information).

The reaction medium plays an important role in the formation of the polymer nanocapsules and controlling their size. For example, the photopolymerization of **1** and **2a** in acetonitrile and chloroform produces polymer nanocapsules with an average diameter of (150 ± 50) nm and (600 ± 100) nm, respectively (Figure 2d). However, the same reaction in *N*,*N*-dimethylformamide yields not only nanocapsules but also rolled or folded thin films, the exact nature of which is under further investigation. The size of the polymer capsules can also be controlled by the length of the dithiol. For example, the longer dithiol **2b** produces the larger nanocapsule **3b** (average diameter (140 ± 40) nm) whereas the shorter dithiol **2c** yields the smaller nanocapsule **3c** (average diameter (50 ± 10) nm) under the same conditions, as revealed by DLS studies.

To understand the mechanism of formation of the polymer nanocapsule, we monitored the photopolymerization reaction by DLS and FTIR spectroscopy. First, no preorganized structure was found in a mixture of **1** and **2a** in methanol before UV irradiation. However, after around 4 minutes of the reaction, particles with an average size of 70 nm suddenly appeared and the particle size quickly reached 105 nm within 3 h and remained essentially the same afterward (see Figure S5 in the Supporting Information). The FTIR spectrum of the product isolated after 3 h of the reaction revealed the presence of unreacted allyl groups which slowly disappeared over the next several hours (see Figure S6 in the Supporting Information).

Although the mechanism of the polymer nanocapsule formation needs further investigation, on the basis of the above observation and general features of the thiol-ene photopolymerization, which is known to follow a free-radical step-growth mechanism,[7] we propose the following mechanism (Figure 3a). 1) At the very early stage of the reaction, the disk-shaped monomers **1** with multiple polymerizable groups at the periphery form dimers and trimers linked by thioether linkages, which further react with each other to grow into 2D oligomeric patches. 2) A 2D oligomeric patch of a certain size starts to bend to reduce its total energy, and the further reaction between the curved oligomeric patches generates a loosely cross-linked hollow sphere. 3) Some of the remaining allyl groups participate in additional thioether bridge formation between neighboring CB[6] units in the shell to produce a highly cross-linked polymer nanocapsule, while the rest of the allyl groups lead to the formation of disulfide loops protruding from the nanocapsule surface (Figure S3 in the Supporting Information). The proposed

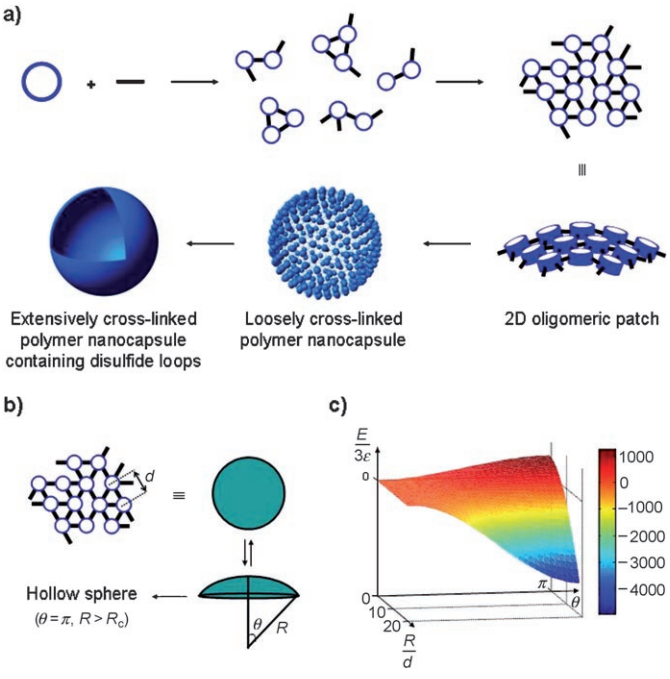

Figure 3. Mechanism and energy profile associated with the nanocapsule formation process. a) Proposed mechanism of the formation of polymer nanocapsules (see also Figure S3 in the Supporting Information). b) Theoretical model for the conversion of a disk (representing a 2D oligomeric patch) into a cap and then a hollow sphere. c) Energy profile ($E/3\varepsilon$) of a cap as a function of θ and R/d, assuming that $\kappa = 150\varepsilon$, $f = 6$, and $R_c = 9d$.

mechanism is similar to the well-accepted mechanism of the formation of vesicles, in which a 2D planar bilayer structure is assumed to be involved before it turns into a vesicle[11] and also shares important characteristics with models for virus self-assembly.[12] The major difference is that covalent bonds are formed between the building units in the lateral directions in the present system.

For a better understanding of the formation process of the hollow sphere, a theoretical study was performed. The 2D oligomeric patch may be regarded as a disk which can be spontaneously transformed into a cap characterized by a radius of curvature R and angle θ (Figure 3b) as a result of thermal fluctuation. The total energy of the cap is given by a sum of three competing contributions: the surface energy, the energy cost to expose the rim, and the bending energy [Eq. (1)], where d is the average distance between neighboring monomers, ε is the average bond energy for a single linkage between neighboring monomer units, f is the number of such linkages per monomer, and κ is the bending modulus largely modulated by flexibility of polymer linkages.

$$E = -\frac{f}{2}\varepsilon\left[\frac{8R^2}{d^2}(1-\cos\theta)-\frac{2\pi R}{d}\sin\theta\right]+4\pi\kappa(1-\cos\theta) \quad (1)$$

Figure 3c shows an energy profile of the cap as a function of θ and R which suggests that, provided the surface energy dominates the bending energy, the disk ($\theta=0$) can spontaneously transform into a hollow sphere ($\theta=\pi$) of a radius larger than a critical value $R_c \approx d\sqrt{\kappa/\varepsilon}$. While the energy dictates fewer, larger spheres to form, the entropy favors more, smaller spheres. The free-energy-minimizing result is the formation of hollow spheres of varying radii distributed by a weighted Gaussian, $P(R) \approx R\exp(-R^2/2\sigma^2)$, with both the mean and variance-like quantity σ in the order of $dC^{1/4}\exp(2\pi\kappa/kT)$, where C is the total concentration of the monomer and k is the Boltzmann constant, according to our theory adapted from reference [11]. This means that changes in the distance between neighboring monomers, polymer stiffness, and solvents affect the average size and size distribution in a very appreciable manner, which is qualitatively in good agreement with the experimental results. The details of the theoretical study will be published elsewhere. The theoretical study also suggests that polymer hollow spheres can be produced from any monomers with a disk-shaped core and multiple polymerizable groups at the periphery leading to polymerization in the lateral directions. Indeed, a preliminary result has shown that the photoreaction of triphenylene derivative **4**, which contains six allyl groups, with dithiol **2a** produces polymer nanocapsule **5a** with an average diameter of (900 ± 120) nm (Figure 2e,f; see the Supporting Information for details).

The polymer nanocapsules can encapsulate guest molecules in their interior. For example, UV irradiation of a mixture of **1** and **2a** in the presence of carboxyfluorescein (CF) followed by dialysis produced a polymer nanocapsule encapsulating carboxyfluorescein (CF@**3a**) with an average diameter of (160 ± 40) nm as confirmed by DLS and SEM studies. The successful encapsulation of the guest was confirmed by a red shift of about 30 nm of the emission band (see Figure S7 in the Supporting Information). No change in the position and intensity of the emission band upon further dialysis for days supports the trapping of the guest molecule. Upon addition of an acid or methyl viologen to a dispersion of CF@**3a**, the fluorescence of the encapsulated dye was quenched instantly or slowly, respectively (see Figure S8 in the Supporting Information), illustrating the size-selective permeability of the nanocapsule shell.

As the polymer capsules are made of a CB[6] derivative which is known to form exceptionally stable host–guest complexes (binding constants over $10^6\,M^{-1}$) with polyamines such as spermine and spermidine,[8] the capsule surface could be easily probed and tailored using host–guest chemistry, that is, noncovalent interactions between the accessible CB[6] units on the surface and polyamine derivatives.[13] For example, the accessible CB[6] cavities on the surface can be quantified by fluorescence spectroscopy using the fluorescein isothiocyanate (FITC)-tagged polyamine **6**, which forms a stable 1:1 host–guest complex with **1**, as a probe. Approximately 85% of the host molecules **1** that constitute the polymer nanocapsules **3a** are accessible to the fluorescent probe, which implies that the nanocapsule shell is essentially single-molecule thick. The strong host–guest interaction ensures near-quantitative binding of the probe to the surface of the polymer capsules when 0.8 equivalents or less (with respect to all the constituent host molecules) of **6** are added to a dispersion of **3a**. A negligible amount of free **6** released from the surface-decorated nanocapsule upon prolonged dialysis confirms the exceptional stability of the noncovalently modified surface. The noncovalent surface modification little affects the shape and size of the polymer nanocapsule. These results thus provide a versatile, noncovalent, and modular approach to the surface modification of the polymer capsules.

To illustrate the potential utility of such surface-decorated polymer nanocapsules, the receptor-mediated endocytosis of polymer nanocapsules decorated with a ligand was studied. Folic acid is a high-affinity ligand for folate receptors, which are tumor markers that are overexpressed in many human tumors.[14] Polymer nanocapsules **3a** decorated with FITC-spermine conjugate ligand **6** (as a fluorescent probe) and folate-spermidine conjugate ligand **7** were prepared in the same way as described above. Their receptor-mediated endocytosis into human oral cancer KB cells was monitored by confocal microscopy. No significant endocytosis was observed for **3a** decorated with only **6**, whereas facile endocytosis was evident for **3a** decorated with both **6** and **7** as illustrated in Figure 4, indicating that this approach may provide a potentially viable approach to targeted drug delivery.

In summary, we have demonstrated a direct approach for the synthesis of polymer nanocapsules without using any preorganized structure, emulsifier, or template that appears to be applicable to any monomers with a flat core and multiple polymerizable groups at the periphery. The embedded host molecules in the shell with exceptionally high binding affinity toward polyamines provide unique opportunities to tailor their surface in a nondestructive and noncovalent manner, making the polymer nanocapsules poten-

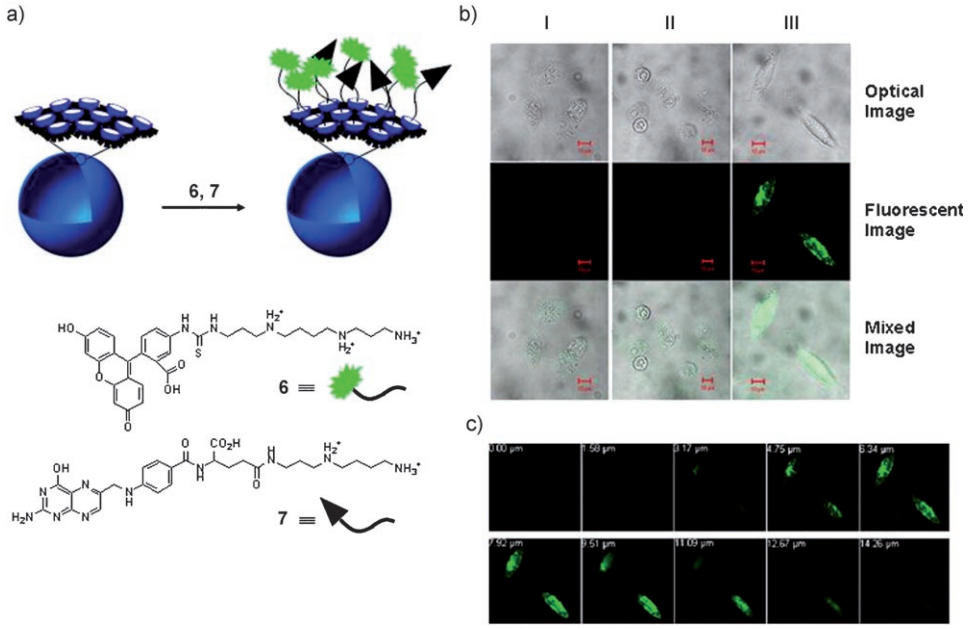

Figure 4. Preparation of surface-decorated polymer nanocapsules and confocal microscopy images of the polymer nanocapsules incorporated into KB cells. a) Schematic representation of the surface modification of polymer nanocapsules through host–guest interactions. b) Confocal micrographs of untreated KB cells (I), cells treated with **3a** decorated with only **6** (II), and cells treated with **3a** decorated with both **6** and **7** (III). Scale bars (red): 10 μm. c) Confocal micrographs taken with different focal depths along the view direction confirm the internalization of **3a** decorated with both **6** and **7** in KB cells (from 0.0 μm (top left) to 14.26 μm (bottom right) in steps of approximately 1.58 μm).

tially useful in many applications including targeted delivery and imaging.

Experimental Section

Polymer nanocapsule **3a**: α,ω-Dithiol **2a** (43.7 mg, 240 μmol) was added to a solution of **1** (10.4 mg, 5.0 μmol) in methanol (10 mL). After purging with N_2, the mixture was irradiated with UV light (254 nm and 300 nm) for 20 h. The product was purified by dialysis using methanol for 2 days to give a homogeneous colloidal solution of polymer nanocapsule **3a** in methanol. Removal of the solvent under reduced pressure, followed by drying in vacuo yielded polymer nanocapsule **3a** (20.5 mg; 87% based on **1**). Elemental analysis (%) calcd for **3a** $[(C_{72}H_{96}N_{24}O_{24})_2(C_6H_{12}O_2S_2)_{31}(CH_4O)_6]_n$: C 44.13, H 6.48, N 7.35, S 21.74; found: C 44.25, H 5.56, N 6.10, S 21.67. The elemental analysis data indicate that polymer nanocapsule **3a** is composed of **1** and **2a** in a ratio of 1:15.5 and contains 87% of the starting material **1**. See the Supporting Information for further characterization of **3a** and other experimental details.

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