Fabrication of Hydrophilic Polymer Nanocontainers by Use of Supramolecular Templates

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Ⓢ *Supporting Information*

ABSTRACT: Polymer-shelled vesicles are prepared by using cyclodextrin vesicles as supramolecular templates and an adamantane-functionalized poly(acrylic acid) additive anchored via host–guest recognition, followed by cross-linking of carboxylic acid groups on the polymer. The polymer-shelled nanocontainers are highly stable and effective for encapsulating small hydrophilic molecules. We also show that a hollow cross-linked polymer cage can be obtained after dissolution of the template vesicles. The size and shell thickness of the polymer cage can be tuned by variation of template size and polymer length.
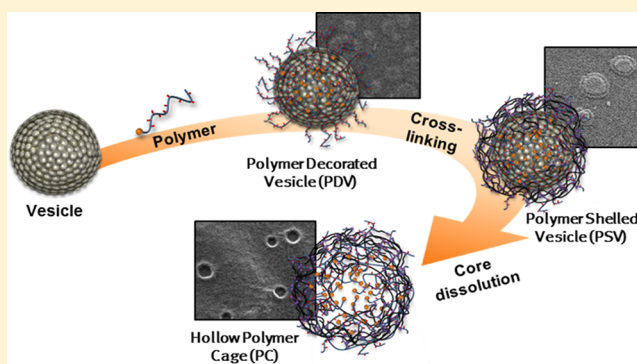
INTRODUCTION

Vesicles are self-assembled nanocontainers consisting of an aqueous core enclosed by one or more bilayers of amphiphilic molecules. Liposomes have received enormous attention in the field of biophysical research as pharmaceutical carriers of great potential because of their intrinsic biocompatibility and ease of preparation.[1] In recent years, many studies have been performed to engineer nanocontainers that enclose a volume with a self-assembled layer of synthetic amphiphiles. Synthetic vesicles provide an opportunity to control the size, structure, and properties of the nanocontainers, which enables the development of biomimetic systems, drug and gene delivery systems, light-harvesting systems, and microreactors.[2] However, major disadvantages of conventional liposomes include their instability and lack of possibilities to tune the transmembrane permeability. There have been numerous attempts to enhance the stability of liposomes by incorporation of polymerizable lipid amphiphiles, which leads to stable cross-linked liposomes.[3] However, installing a polymerizable residue to an amphiphile without changing its packing parameter is synthetically cumbersome. Hydrophilic polymers such as poly(ethylene glycol) (PEG)[4] and poly(N-isopropylacrylamide)[5] can be added to liposomes to improve the stability and modify their surface. However, these polymer residues dissociate easily from the liposome surface, thereby regenerating the unstable state of the parent liposome.[6,7] In 2007, Nguyen and co-workers[8] reported a drop-in strategy to prepare polymer-caged liposomes using conventional liposomes and a cholesterol-functionalized poly(acrylic acid) additive. Nevertheless, a controlled method to design nanocapsules from a single homopolymer is still challenging. In particular, two techniques have been used to engineer polymeric vesicles or polymeric capsules: (1) self-assembly of tailor-made amphiphilic block copolymers and (2) layer-by-layer (LbL) assembly of polyelectrolytes on microparticles.[9] Wooley and co-workers[10] reported a polymer-shelled hollow nanocage from poly(isoprene-b-acrylic acid) block copolymer degrading the core of shell cross-linked knedels (SCK). Caruso and co-workers[11] synthesized polymer-shelled microparticles via LbL deposition of polymers on templates made of either silica or poly(methacrylic acid). Fabrication of polymer hollow spheres using vesicles as a template has been reported by Hotz and Meier,[12] who designed a polymer container by cross-linking polymerization of methacrylate monomers trapped in the bilayer of dimethyldioctadecylammonium chloride (DODAC) vesicles. Herein, we demonstrate a simple and effective way to fabricate highly stable nanosized polymer-shelled vesicles (PSV) by use of cyclodextrin vesicles (CDV) as supramolecular templates and adamantane-terminated poly(acrylic acid) (Ad-PAA) as building blocks (Figure 1). We show that a robust hollow polymer cage (PC) can be prepared simply by washing out the template from the PSV (Figure 2). Importantly, the size and shell thickness of the polymer cage can be tuned by variation of template size and polymer length.

In recent years we have reported the formation of unilamellar vesicles (100−150 nm) composed of amphiphilic cyclodextrins (Figure 1) and the molecular recognition of various guest molecules at the surface of such self-assembled nanocontainers.[13] An important advantage of CDV in contrast
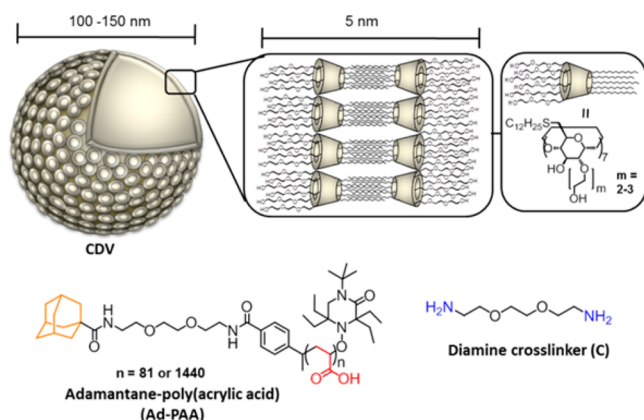
Figure 1. Schematic representation of cyclodextrin vesicles (CDV) and molecular structures of adamantane-terminated poly(acrylic acid) (Ad-PAA) and diamine cross-linker (C).
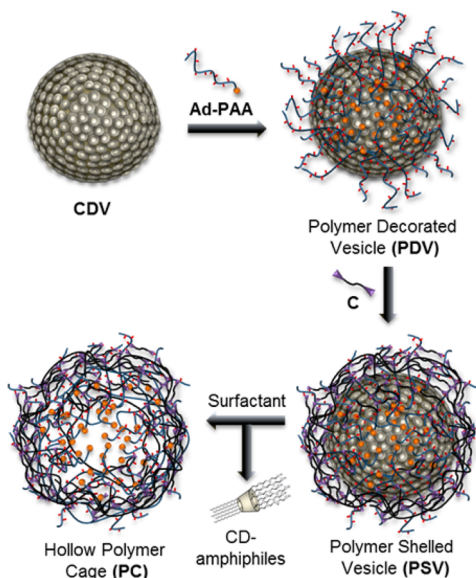

Figure 2. Stepwise representation of synthesis of PDV, PSV, and PC by use of CDV as a template.

with conventional liposomes is the fact that CDV can selectively form inclusion complexes with hydrophobic guest molecules (such as adamantanes, *t*-butylbenzenes, and azobenzenes) and hence can be decorated with functional guest molecules such as oligonucleotides, carbohydrates, and peptides simply by mixing the host vesicles with the desired mixture of guest-tagged biomolecules.[14] By using this host–guest interaction of adamantane and *β*-cyclodextrin (binding constant for single interaction $K_a \sim 10^4$ M^{-1}), the bare CDV can easily be decorated with an adamantane-terminated poly(acrylic acid) and then further stabilized by cross-linking of the poly(acrylic acid). The resulting PSV are extremely stable and almost impermeable, even if the template is removed by washing.

■ RESULTS AND DISCUSSION

A narrowly dispersed adamantane-terminated poly(acrylic acid) [M_n = 11.1 kDa, M_w/M_n = 1.2, degree of polymerization (DP) ∼ 81] was synthesized by nitroxide-mediated polymerization of *tert*-butyl acrylate, followed by hydrolysis of *tert*-butyl groups with trifluoroacetic acid (see Supporting Information for

details). We used the adamantane group to immobilize the polymer at the surface of CDV through host–guest interaction with the cyclodextrin cavity. The advantage of using poly-(acrylic acid) is to have easily cross-linkable carboxylic acid groups. Amphiphilic *β*-cyclodextrin was synthesized as described in the literature.[13b] Unilamellar CDV were prepared by extrusion in buffer [20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 0.15 M NaCl] at pH 7.4. The average diameter of CDV is 128 nm according to dynamic light scattering (DLS) (Figure 3A, black trace). To
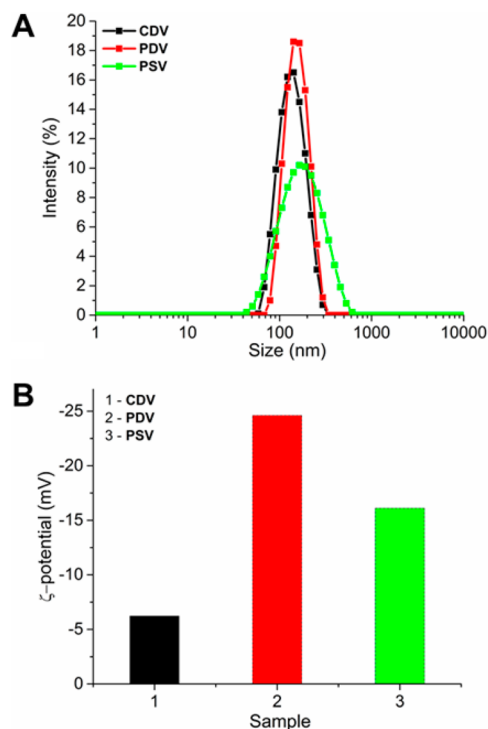

Figure 3. (A) Size distribution according to dynamic light scattering. (B) ζ-potential of CDV, PDV, and PSV. [CDV] = 100 μM, [Ad-PAA] = 25 μM, [C] = 0.5 mM in 20 mM HEPES buffer (pH 7.4).

investigate the characteristics of the resulting polymer-decorated vesicle (PDV), 0.025 mM solution of Ad-PAA was added to 0.1 mM solution of CDV. It can be assumed that Ad-PAA does not permeate the vesicle membrane due to the hydrophilicity of the poly(acrylic acid) moiety. Hence, at these concentrations close to 50% of cyclodextrin cavities on the outside surface of the CDV will be occupied with Ad-PAA and the concentration of free polymer in the solution is negligible. According to DLS, particles with an increased average diameter of 142 nm were observed (Figure 3A, red trace), indicating the formation of homogeneous PDV. Cross-linking of the acrylic acid moieties (0.25 equiv of total carboxylic acid for a theoretical 50% cross-linking) was carried out with 2,2′-(ethylenedioxy)bis(ethyleneamine) (C) in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) followed by a dialysis to remove the byproducts. The average hydrodynamic diameter of PSV increased to 150 nm (Figure 3A, green trace). The ζ-potential measurements were performed to corroborate the DLS measurements. As shown in Figure 3B, the ζ-potential of CDV is ca. −6.2 mV at pH 7.4. The negative surface potential arises due to the oligo(ethylene glycol) residues on the vesicle surface.[13b] The surface potential decreases from ca. −6.2 to −24.6 mV after addition of Ad-PAA.

The decrease of surface potential of PDV is due to the presence of poly(acrylic acid) residues at the surface of the vesicles. The ζ-potential of PSV increases to −16.1 mV, which reflects significant cross-linking of acrylic acid moieties.

To investigate the morphology of PDV and PSV, field emission scanning electron microscopy (FESEM) (secondary electron imaging) was performed on air-dried samples (Figure 4). The particle size observed in FESEM is consistent with the
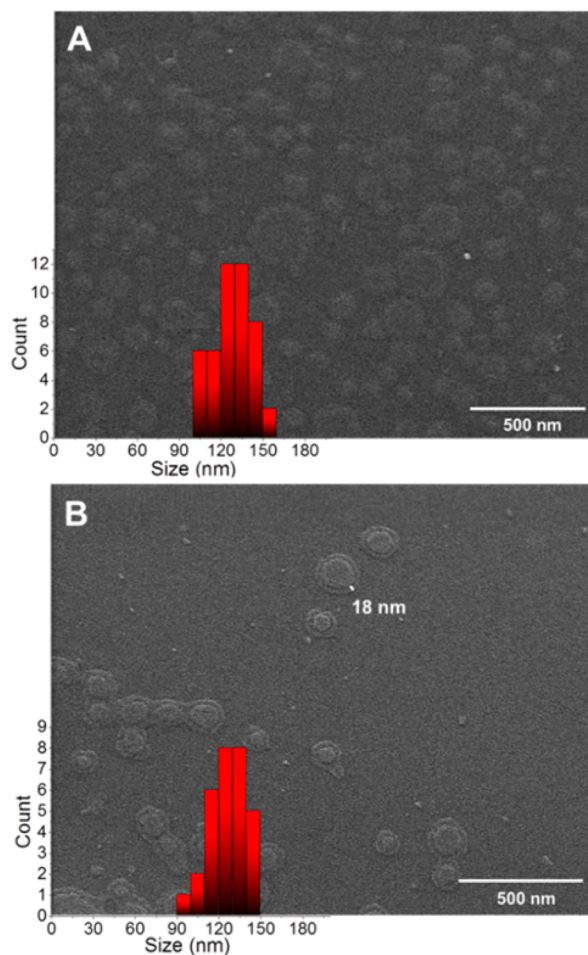

Figure 4. (A) FESEM image of PDV. (B) FESEM image of PSV. (Inset) Size distribution according to SEM images. [CDV] = 1 mM, [Ad-PAA] = 250 μM, [C] = 5 mM in 20 mM HEPES buffer (pH 7.4).

diameters obtained by DLS. CDV are composed of very soft and flexible bilayer membranes and hence it is difficult to image them by FESEM in the air-dried state. The average diameter observed in FESEM is smaller than that found in DLS, which is likely a result of sample preparation (i.e., drying). Most interestingly, the FESEM image of PSV clearly shows the polymer coating on the surface of the nanostructures (Figure 4B). The thickness of the polymer shell is around 18 nm, which matches the increase in diameter of PSV from bare CDV observed in DLS measurements.

The PSV are remarkably stable. Their spherical structure was fully preserved after addition of excess Triton X-100. Upon addition of 2% Triton X-100 to 0.1 mM CDV solution, the vesicles are instantaneously solubilized and micelles are predominantly observed by DLS (Figure 5A, pink trace). Upon addition of the same amount of Triton X-100 to PSV followed by dialysis, a hollow PC can be obtained (Figure 5A,
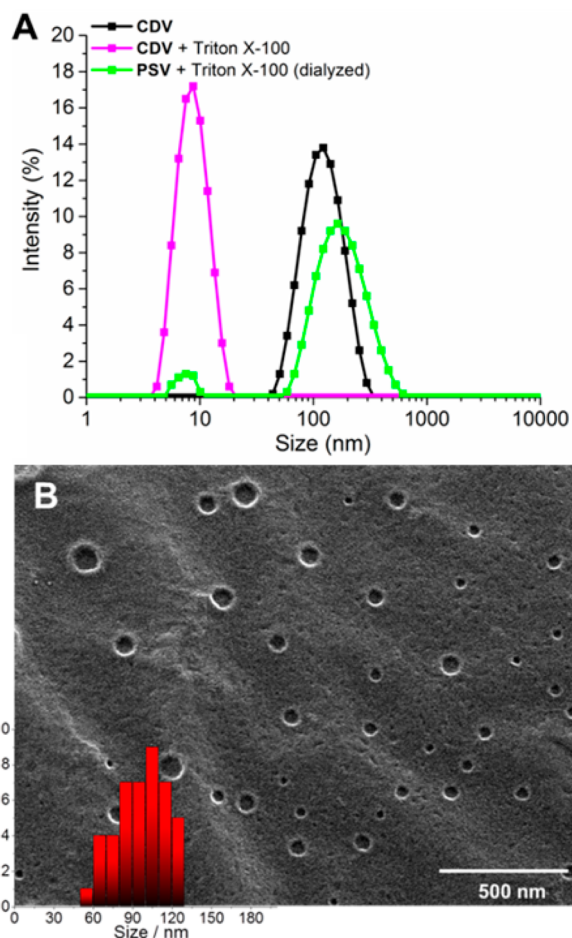

Figure 5. (A) Size distribution according to dynamic light scattering. [CDV] = 100 μM, [Ad-PAA] = 25 μM, [C] = 0.5 mM. (B) FESEM image of PC. (Inset) Size distribution according to SEM images. [CDV] = 1 mM, [Ad-PAA] = 250 μM, [C] = 5 mM in 20 mM HEPES buffer (pH 7.4).

green trace). The cross-linked poly(acrylic acid) shell is obviously resistant to the surfactant and we assume that during the dialysis all cyclodextrin amphiphiles are washed out with Triton X-100. The shape of the PC was investigated by FESEM (Figure 5B). The size of the PC calculated from the FESEM image is smaller than the diameter observed by DLS. The difference in diameter arises due to drying of the hollow polymer capsules during sample preparation.

In order to understand the effect of pH on PDV and PSV, DLS measurements were performed in a pH range of pH 4−12. Neither of the nanostructures is affected at higher pH due to the increasing deprotonation of poly(acrylic acid) residues. However, at pH 6, PDV start aggregating due to diminishing electrostatic repulsion (protonation of acrylate moieties) among the particles, while PSV remain unaffected until pH 4, which clearly indicates the presence of N-alkyl acrylamide cross-links at the PSV surface (Figure S1, Supporting Information).

An important property for drug carrier vesicles is low permeability. We investigated the transmembrane permeability of PDV and PSV from leakage experiments of sulforhodamine B encapsulated in vesicles. To this end, CDV (1 mM) were prepared in a solution of sulforhodamine B at self-quenching concentration (20 mM) and Ad-PAA was added to the vesicle solution. To separate the dye-encapsulated PDV from free dye

in solution, gel filtration was performed on a Sephadex G-50 column. Dye-encapsulated PSV were obtained by cross-linking the carboxylic acid groups on PDV before the gel-filtration separation of dye. After gel filtration, the vesicles were diluted to ca. 0.1 mM total cyclodextrin amphiphile concentration, and the sulforhodamine B fluorescence intensity was measured over time and finally after the addition of Triton X-100. In our previous studies, we have shown that the dye leaks out within 5−10 min after gel filtration in the case of bare CDV due to the presence of loosely packed amphiphilic cyclodextrin in the membrane.[13b,15] Triton X-100 solubilizes the vesicle core and leads to maximum fluorescence intensity due to complete relief of self-quenching (see Supporting Information for fluorescence spectra). As shown in Figure 6, the dye leaks out faster in the
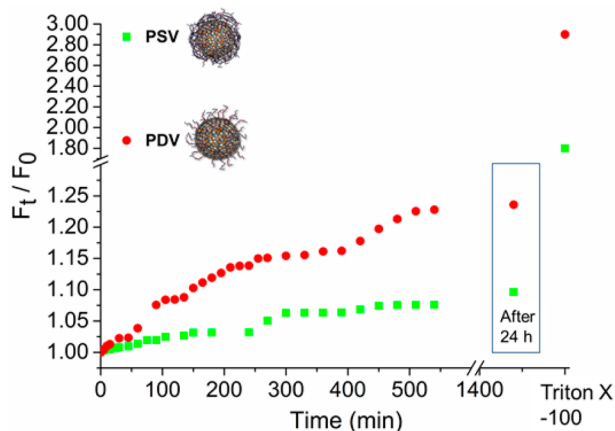

Figure 6. Leakage of sulforhodamine B from PDV and PSV. Normalized fluorescence intensity F_t/F_0 is plotted with time and after addition of Triton X-100.

case of PDV than PSV due to the steric barrier provided by the cross-linked polymer shell. Remarkably, only a minute leakage of sulforhodamine B (percentage of leakage ~8%) was observed from PSV after 8 h. Leakage in the case of PDV is ~14% after 8 h, which is attributed to the fact that, due to the dynamic nature of the monovalent host−guest interaction, the non-cross-linked Ad-PAA can temporarily dissociate from the surface and lead to increased leakage from the PDV compared to PSV. In this sense, the cross-linked polymer shell of PSV reduces the leakage of molecules entrapped in the interior and prolongs the vesicle stability due to multivalent host−guest inclusion of adamantane and β-cyclodextrin at the vesicle surface.

Importantly, the size and shell thickness of PSV as well as PC can be adjusted either by using Ad-PAA of different length or by varying the size of the template vesicles. To synthesize a larger polymer-shelled vesicle, a relatively long adamantane-functionalized poly(acrylic acid) derivative was used (Ad-PAA; M_n = 185 kDa, M_w/M_n = 1.5, DP ~ 1440). The corresponding polymer-decorated vesicles (PDV), polymer-shelled vesicles (PSV), and polymer capsules (PC) were prepared in exactly the same way mentioned above. It can be seen from the DLS data in Figure 7A (red trace) that the average diameter of PDV increased to 210 nm after addition of Ad-PAA to the template CDV (average diameter =128 nm) solution. After cross-linking of carboxylic acid groups at the surface of PDV, the average diameter of PSV slightly increased to 215 nm (Figure 7A, green trace). The DLS data for PDV and PSV thus clearly indicated
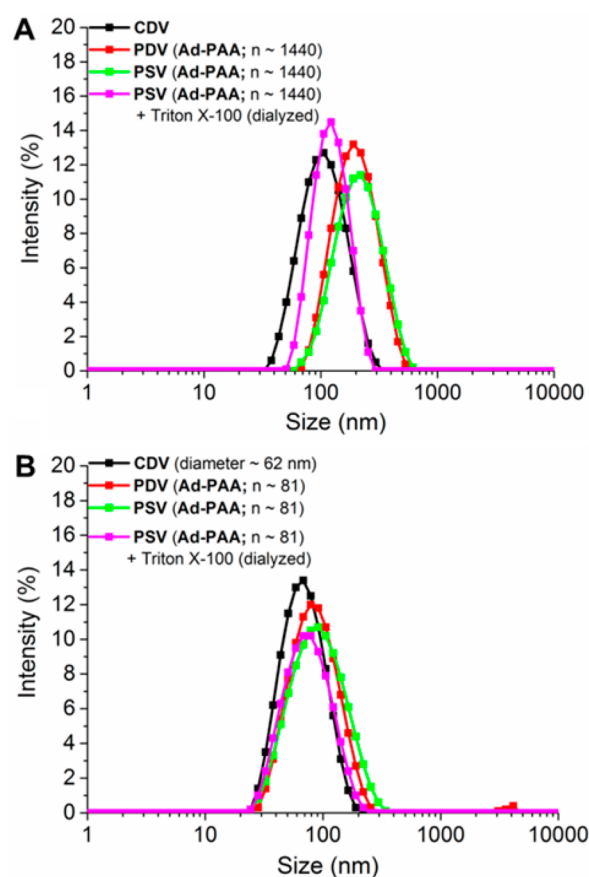

Figure 7. Size distribution according to dynamic light scattering. (A) Size distribution of CDV, PDV, PSV, and PC synthesized from Ad-PAA with DP ~ 1440. (B) Size distribution of CDV, PDV, PSV, and PC synthesized from CDV with a diameter of 62 nm and Ad-PAA with DP ~ 81. [CDV] = 100 μM, [Ad-PAA] = 25 μM, [C] = 0.5 (in panel A) and 6 mM (in panel B), [Triton X-100] = 2% (w/v) in 20 mM HEPES buffer with 0.15 M NaCl (pH 7.4).

that the longer Ad-PAA (DP ~ 1440) creates a much thicker shell on the CDV surface than the shorter Ad-PAA (DP ~ 81). PC with an average diameter of 205 nm were obtained after the amphiphilic β-CD was dialyzed out from these PSV in the presence of Triton X-100 (Figure 7A, pink trace). The release of sulforhodamine B from dye-loaded PSV was monitored over time by fluorescence spectroscopy. Surprisingly, the rate of leakage from PSV with a thicker polymer shell is only slightly lower than that of PSV with a thinner polymer shell (Figure S3A, Supporting Information). The percentage of leakage in case of the PSV prepared with Ad-PAA (DP ~ 1440) is ~6% after 400 min. Finally it can be observed from the FESEM image (Figure S3B, Supporting Information) that PC with an average diameter of 185 nm was prepared by use of the longer Ad-PAA.

Moreover, smaller PSV were prepared by using a CDV template with an average diameter of 62 nm (Figure 7B, black trace). Small CDV were prepared by extruding the vesicle solution through a polycarbonate membrane with 50 nm pore size. The average diameter of the PDV obtained by decorating the small CDV with Ad-PAA (DP ~ 81) is only 77 nm (Figure 7B, red trace). Upon cross-linking of the carboxylic acid groups at the periphery of the PDV, PSV with an average diameter of 79 nm result (Figure 7B, green trace). Finally, small PC with

average diameter of 75 nm were obtained by washing out the CDV template with Triton X-100 and dialysis.

CONCLUSION

In summary, we present a new method to make hydrophilic nanocontainers based on a soft supramolecular template. These containers consist of polymer-shelled vesicles, which were prepared by self-assembly from cyclodextrin vesicles (CDV) and an adamantane-functionalized poly(acrylic acid) via host–guest complexation followed by cross-linking of carboxylic acid groups on the polymer. These PSV are highly stable and much less permeable than the bare CDV. Most interestingly, our results show that a hollow polymer cage (PC) can be obtained by washing out the CDV template from the PSV. The PC diameter can be tuned by using smaller or larger vesicles as templates, and the polymer shell thickness can be tuned by using a shorter or longer polymer. It is hoped that the nanocontainers reported here can be developed into versatile drug delivery systems.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional text describing materials and methods and synthesis and analysis of polymers; 12 figures showing size distribution of PDV and PSV at different pH according to DLS, encapsulation of sulforhodamine in vesicles with PDV and PSV, leakage of sulforhodamine B from PSV and PC, FESEM image of PC, size distribution of sulforhodamine B, and ^{1}H and ^{13}C NMR spectra of **1**–**4**; one scheme showing synthesis of Ad-PAA. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

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