Charge and Size Selective Molecular Transport by Amphiphilic Organic Nanotubes

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S *Supporting Information*

ABSTRACT: Amphiphilic constructs with accessible, nanometer-size cavities can enable selective encapsulation, separation, and purification of nanomaterials and biomacromolecules on a similar length scale. We have developed a new method for the fabrication of amphiphilic organic nanotubes from multicomponent bottlebrush copolymers with triblock terpolymer side chains. The obtained nanotubes were demonstrated to be very effective and highly selective carriers for positively charged molecules and nanometer-size macromolecules by means of liquid—liquid extractions. Unprecedented discrimination between dendrimers with about 2 nm size differential was achieved.

Figure 1. Molecular templating of bottlebrush copolymers with triblock terpolymer side chains to produce tubular nanostructures containing functional groups on the interior.

Amphiphilic molecules comprise a broad range of compounds containing segments with distinctly different solubility characteristics. Segregation of chemically similar groups in selective solvents often leads to the formation of compartmentalized aggregates with outer and inner domains that can be used for encapsulation and separation of solutes for a variety of applications. At one end of spectrum, there are surfactants and their macromolecular analogues, amphiphilic block copolymers, which spontaneously self-assemble into micelles when exposed to a discriminating environment.[1] These robust aggregates have been used since the dawn of time as detergents and, most recently, as drug delivery vehicles and molecular shuttles.[2] However, the dynamic and flexible nature of micellar aggregates renders them rather indiscriminating toward the shape and size of encapsulated molecules. At the other end of amphiphile spectrum, there are well-defined molecular cages, for example, cyclodextrin,[3] calixarene,[4] and cucurbituril,[5] which possess an unmatched ability to selectively bind/encapsulate molecules of certain shape and size. However, such superb selectivity is only reserved for a limited number of molecules with subnanometer dimensions.

In recent years, there has been a flurry of activity focused on bridging the gap between the two extremes and developing robust and tunable amphiphiles for *selective* encapsulation. An important outcome of these research efforts has been branched amphiphilic macromolecules, such as dendrimers,[6] hyperbranched[7] and star polymers,[8] which fashion unimolecular micelles in selective solvents. These macromolecules, which often contain a preformed core—shell type architecture and do not rely on self-assembly, can exhibit selective guest binding, mostly based on electrostatic interactions.[6c,7a,9] Well-defined cavities inside dendrimers can also serve as selective pockets for capturing guest molecules with subnanometer dimensions.[9d,10] On the other hand, encapsulation, separation, and purification of nanomaterials and biomacromolecules, which has become an increasingly important challenge with
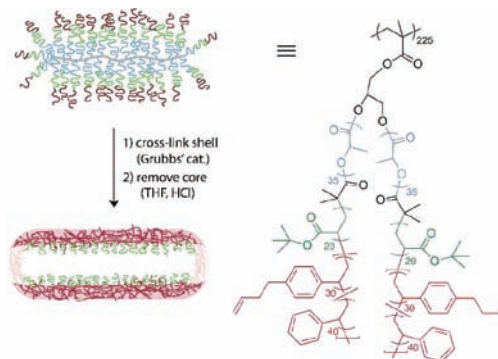
the advent of nanotechnology and nanomedicine, requires the availability of molecules with well-defined, accessible, nanometer-size cavities. In this communication, we report a new class of organic amphiphilic nanostructures, composed of nanotubes with a hydrophobic exterior and a hydrophilic interior, that exhibit guest discrimination at a nanometer length scale. We demonstrate that a well-defined interior surface chemistry and cavity diameter allow for an unprecedented selectivity toward encapsulation and extraction, based on charge, size, and architecture of guest molecules.

Bottlebrush copolymers, or molecular brushes, are a class of highly branched macromolecules with a comb-like architecture, where shorter polymeric side chains are densely grafted along a longer polymeric backbone.[11] As we and others have previously demonstrated, organic nanotubes can be prepared by molecular templating of multicomponent bottlebrush copolymers with diblock copolymer side chains.[12] Cylindrical shape and dimensions of bottlebrush copolymers in solution are preserved during templating, which is achieved by selective shell cross-linking and core degradation. Such a nanotube fabrication method, inspired by Wooley's work on shell-cross-linked micellar nanoparticles,[13] combines advantages of solid templating methods and self-assembly approaches, producing organic nanotubes with well-defined dimensions in a conveniently scalable process.

To render nanotubes amphiphilic, we employed methods previously developed in our group[12c,14] to synthesize bottlebrush copolymers with triblock terpolymer side chains, which allowed for an independent control of inner and outer surface properties of the nanotubes (Figure 1). Thus, every side chain was composed
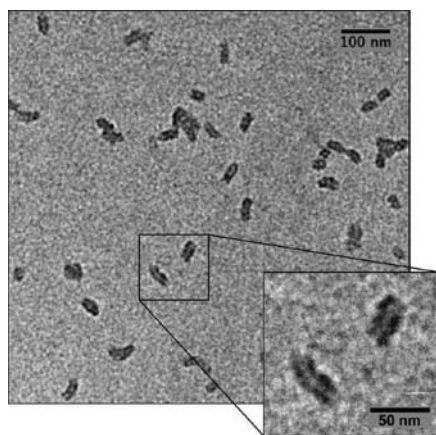
Figure 2. TEM image of organic nanotubes deposited from a chloroform solution.

Figure 3. Biphasic mixtures of aqueous solutions of ionic dyes and chloroform solutions of nanotubes after shaking for 5 min: (A) [MB]/[pure chloroform], (B) [MB]/[organic nanotubes without PAA layer], (C) [MB]/[amphiphilic nanotubes], (D) [ARS]/[pure chloroform], (E) [ARS]/[amphiphilic nanotubes].

of an inner degradable block (polylactide, PLA), an outer cross-linkable block (poly(styrene-*ran*-(4-(3-butenyl)styrene))), and a middle functional block (poly(*t*-butyl acrylate), P*t*BA) to dictate nanotube interior surface chemistry. Porous cylindrical nanoparticles were obtained by shell cross-linking of bottlebrush copolymers with Grubbs' catalyst and subsequent removal of polylactide core by acidic hydrolysis. The latter process also deprotected *t*-butyl groups and exposed poly(acrylic acid) coating on the nanotube interior. Transmission electron microscopy (TEM) analysis revealed that the shape and size of organic nanoparticles were mostly preserved during hydrolytic removal of the core, thus, generating 36 nm-long tubular nanostructures containing an internal cavity with an average diameter of 6 nm (Figure 2). Hydrodynamic diameter (D_h) of nanotubes in chloroform was measured to be 39 nm by dynamic light scattering (DLS). The fact that average nanotube dimensions obtained from TEM (dried sample) and DLS (in solution) analyses are consistent with each other suggests that the cross-linked nanotube shell is quite rigid and does not allow significant swelling in solution. The obtained nanoparticles were easily dispersed in chloroform and other organic solvents, indicating that hydrophilic PAA brushes were on the inside, in general agreement with overall molecular design and block connectivity in the bottlebrush copolymer precursor. Every nanotube contained approximately 9000 carboxylic acid groups, on average, as estimated from NMR analysis of the bottlebrush copolymer precursors.

We performed a series of liquid−liquid extraction experiments to evaluate selective encapsulation capability of amphiphilic organic nanotubes (Figure 3). Methylene Blue (MB, a cationic dye) and Alizarin Red S (ARS, an anionic dye) were chosen as water-soluble and chloroform-insoluble dye molecules with a similar size and opposite charges. Initially, in a biphasic system, the dye is present in the aqueous phase (0.15 mM), while nanotubes are dissolved in the chloroform layer (1 mg/mL). After rigorously shaking the mixture for a few minutes, all MB dye was transferred to the organic phase, leaving the aqueous phase completely clear (Figure 3C). Remarkably, the phase transfer process was very rapid, eliminating the need for prolonged stirring. On the other hand, no transfer was observed when a negatively charged ARS dye was dissolved in the aqueous phase (Figure 3E). Additionally, no MB or ARS dye transfer was observed in the absence of organic nanotubes in the chloroform layer. We contend that electrostatic interactions between a positively charged dye (MB) and a negatively charged
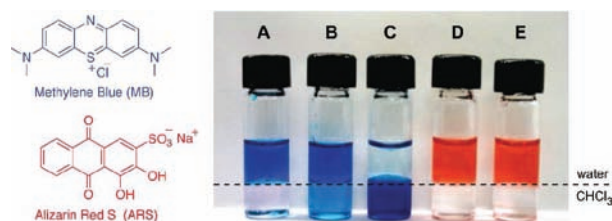
nanotube interior are driving the dye uptake inside the nanotubes, thus, facilitating their transport to the organic phase. To evaluate the loading capacity of amphiphilic organic nanotubes, we performed extraction experiments with a concentrated MB solution (0.6 mM). Under these conditions, nanotube carriers were saturated, and only partial dye transfer was observed. Approximately 4500 dye molecules were uptaken into every nanotube, as measured by UV−vis absorption of the aqueous layer before and after the uptake. This loading corresponds to about one MB dye molecule per two carboxylic acid groups inside the nanotubes. Assuming nanotube dimensions in a chloroform solution are approximately the same as those obtained from TEM images of dried samples (i.e., no swelling), the concentration of MB molecules inside a nanotube can be calculated to be 6.5 M! A variety of other water-soluble and positively charged dyes, such as Ethidium Bromide and Safranin, could also be transferred to the organic phase by amphiphilic nanotubes, suggesting a broad applicability of this approach. The reversible nature of MB encapsulation has been demonstrated by extraction from aqueous solutions with different pH values (Supporting Information).

The importance of poly(acrylic acid) coating on the interior surface of nanotubes was established by control experiments carried out with organic nanotubes synthesized from bottlebrush copolymers that do not contain a poly(*t*BA) block in the middle of each branch. Such nanotubes have significantly fewer carboxylic acid groups on the inside (450 carboxylic acid groups per nanotube), produced as end-groups after PLA degradation. When extraction was carried out by using nanotubes without PAA lined interior, only partial transfer of MB into the organic phase was observed (Figure 3B). From UV−vis absorption, we have calculated that approximately 90 MB molecules were imbibed into every nanotube. Again, no dye transfer was observed when ARS solution was used. Thus, the presence of poly(acrylic acid) coating on the interior surface of nanotubes appears to be essential for producing a sponge-like effect.

Nanometer-size cavity of the fabricated amphiphilic nanotubes can be also utilized for encapsulation of much larger guest molecules whose dimensions span the range of 1−5 nm. For proof-of-principle size-exclusion experiments, we utilized poly(amido amine) (PAMAM) dendrimers as model molecules with nanometer dimensions. These amine-end-capped dendrimers are soluble in water, but insoluble in organic solvents, such as chloroform. In particular, we chose generation 2 (G2) and 4 (G4) dendrimers, whose hydrodynamic diameters in water were measured to be 2.8 and 4.3 nm, respectively, by DLS. Dendrimers were covalently tagged with a Rhodamine B dye and purified by dialysis to allow for an easy detection of the phase transfer process. Figure 4
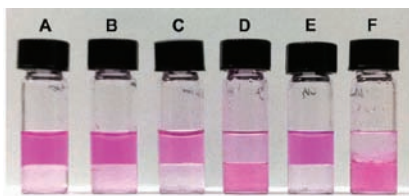
Figure 4. Biphasic mixtures of aqueous solutions of ionic macromolecules and chloroform solutions of nanotubes after shaking for 5 min: (A) [G4]/[pure chloroform], (B) [G4]/[amphiphilic nanotubes], (C) [G2]/[pure chloroform], (D) [G2]/[amphiphilic nanotubes], (E) [PNAA]/[pure chloroform], (F) [PNAA]/[amphiphilic nanotubes].

illustrates the results of selective extractions of PAMAM dendrimers from an aqueous phase (20 μM) into chloroform by amphiphilic organic nanotubes (1 mg/mL). Remarkably, G2 dendrimers, initially dissolved in water, are completely transferred to the organic phase containing nanotubes by rigorously shaking the biphasic mixture for a few minutes. In a striking contrast, G4 dendrimers remain in the aqueous phase, indicating no uptake by the nanotubes. Control experiments in the absence of nanotubes showed no transfer to the organic phase. Maximum loading capacity for G2 dendrimers was calculated to be 450 molecules per nanotube. We attribute such a dramatic size selectivity to the presence of a well-defined internal cavity in the utilized nanotubes.

Dendrimers are compact, highly branched macromolecules with limited degrees of conformational freedom.[15] On the other hand, linear polymers can undergo chain stretching to squeeze into smaller cavities, as long as there is an enthalpic driving force that compensates for the loss of entropy upon chain stretching. We synthesized a linear poly(N-(2-aminoethyl)acrylamide) (PNAA) by RAFT polymerization to contain 160 repeat units. The chemical structure of PNAA is similar to that of PAMAM dendrimers, and D_h of the linear polymer in water was measured to be 4.6 nm, in close proximity to dimensions of a G4 dendrimer. PNAA was covalently labeled with a Rhodamine B dye and dissolved in an aqueous solution (20 μM). Despite having similar dimensions and chemical structure to G4, the linear polymer was quickly transferred to the organic phase by amphiphilic nanotubes when a biphasic mixture was shaken for a few minutes (Figure 4E,F). PNAA by itself is completely insoluble in chloroform, and thus, the results indicate that linear polymer chains are able to penetrate into organic nanotubes, presumably with accompanying chain stretching. The driving force for this uptake is the interaction between carboxylic acid groups on the nanotube interior and pendant amine groups of the polymer (see Supporting Information for more discussion).

In summary, we have demonstrated that amphiphilic organic nanotubes can be utilized as highly discriminating hosts for small and large guest molecules. Organic nanotubes with well-defined dimensions were synthesized from bottlebrush copolymers with triblock terpolymer side chains. Tubular objects were prepared to contain a hydrophobic exterior and an internal cavity coated with hydrophilic, poly(acrylic acid) chains. Such amphiphilic nanostructures were very effective at transporting positively charged dyes from an aqueous phase to chloroform, while not allowing any transfer of negatively charged dye molecules. In addition, the nanotubes showed a remarkable size selectivity in the transport of larger organic guests. Thus, G2 PAMAM dendrimers were effectively transferred from water to chloroform by organic nanotubes, while G4 dendrimers were completely rejected. Such

unprecedented discrimination of molecules with only ∼2 nm size difference was attributed to the presence of well-defined internal cavities in the host organic nanostructures. Linear PNAA polymers of sizes similar to G4 were also imbibed into nanotubes, due to the ability of linear polymers to adapt to different encapsulating microenvironments by undergoing conformational and shape changes. Amphiphilic organic nanotubes and proof-of-principle extraction experiments described in this paper provide a foundation for developing a highly selective platform for encapsulation, extraction, and separations of nanomaterials and biomacromolecules based on charge, size, and shape. We are currently working on establishing the scope and limitations of this new nanotube-based approach.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Complete ref 6d, details on nanotube synthesis, reversible extraction experiments, uptake mechanism discussion and the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) (a) Lindman, B.; Alexandridis, P. *Amphiphilic Block Copolymers: Self-Assembly and Applications*, 1st ed.; Elsevier: Amsterdam ; New York, 2000; p 435; (b) Blanazs, A.; Armes, S. P.; Ryan, A. J. *Macromol. Rapid Commun.* **2009**, 30, 267. (c) Jiwpanich, S.; Ryu, J. H.; Bickerton, S.; Thayumanavan, S. *J. Am. Chem. Soc.* **2010**, 132, 10683. (d) Klaikherd, A.; Nagamani, C.; Thayumanavan, S. *J. Am. Chem. Soc.* **2009**, 131, 4830.

(2) (a) Adams, M. L.; Lavasanifar, A.; Kwon, G. S. *J. Pharm. Sci.* **2003**, 92, 1343. (b) He, Y. Y.; Lodge, T. P. *J. Am. Chem. Soc.* **2006**, 128, 12666. (c) Basu, S.; Vutukuri, D. R.; Thayumanavan, S. *J. Am. Chem. Soc.* **2005**, 127, 16794. (d) Kale, T. S.; Klaikherd, A.; Popere, B.; Thayumanavan, S. *Langmuir* **2009**, 25, 9660. (e) Savariar, E. N.; Aathimanikandan, S. V.; Thayumanavan, S. *J. Am. Chem. Soc.* **2006**, 128, 16224.

(3) Wenz, G. *Angew. Chem., Int. Ed. Engl.* **1994**, 33, 803.

(4) Gutsche, C. D. *Acc. Chem. Res.* **1983**, 16, 161.

(5) Lagona, J.; Mukhopadhyay, P.; Chakrabarti, S.; Isaacs, L. *Angew. Chem., Int. Ed.* **2005**, 44, 4844.

(6) (a) Aathimanikandan, S. V.; Savariar, E. N.; Thayumanavan, S. *J. Am. Chem. Soc.* **2005**, 127, 14922. (b) Ghosh, S. K.; Kawaguchi, S.; Jinbo, Y.; Izumi, Y.; Yamaguchi, K.; Taniguchi, T.; Nagai, K.; Koyama, K. *Macromolecules* **2003**, 36, 9162. (c) Gomez-Escudero, A.; Azagarsamy, M. A.; Theddu, N.; Vachet, R. W.; Thayumanavan, S. *J. Am. Chem. Soc.* **2008**, 130, 11156. (d) Percec, V.; et al. *Science* **2010**, 328, 1009. (e) Vutukuri, D. R.; Basu, S.; Thayumanavan, S. *J. Am. Chem. Soc.* **2004**, 126, 15636. (f) Azagarsamy, M. A.; Sokkalingam, P.; Thayumanavan, S. *J. Am. Chem. Soc.* **2009**, 131, 14184. (g) Cooper, A. I.; Londono, J. D.; Wignall, G.; McClain, J. B.; Samulski, E. T.; Lin, J. S.; Dobrynin, A.; Rubinstein, M.; Burke, A. L. C.; Frechet, J. M. J.; DeSimone, J. M. *Nature* **1997**, 389, 368. (h) Zimmerman, S. C.; Quinn, J. R.; Burakowska, E.; Haag, R. *Angew. Chem., Int. Ed.* **2007**, 46, 8164.

(7) (a) Burakowska, E.; Quinn, J. R.; Zimmerman, S. C.; Haag, R. *J. Am. Chem. Soc.* **2009**, 131, 10574. (b) Irfan, M.; Seiler, M. *Ind. Eng. Chem. Res.* **2010**, 49, 1169. (c) Kitajyo, Y.; Nawa, Y.; Tamaki, M.; Tani, H.; Takahashi, K.; Kaga, H.; Satoh, T.; Kakuchi, T. *Polymer* **2007**, 48, 4683. (d) Satoh, T. *Soft Matter* **2009**, 5, 1972. (e) Stiriba, S. E.; Kautz, H.;

16728

dx.doi.org/10.1021/ja204296v |*J. Am. Chem. Soc.* 2011, 133, 16726–16729

Frey, H. *J. Am. Chem. Soc.* **2002**, *124*, 9698. (f) Sunder, A.; Kramer, M.; Hanselmann, R.; Mulhaupt, R.; Frey, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 3552.

(8) (a) Meier, M. A. R.; Gohy, J. F.; Fustin, C. A.; Schubert, U. S. *J. Am. Chem. Soc.* **2004**, *126*, 11517. (b) Park, J.; Moon, M.; Seo, M.; Choi, H.; Kim, S. Y. *Macromolecules* **2010**, *43*, 8304.

(9) (a) Wan, D. C.; Pu, H. T.; Cai, X. Y. *Macromolecules* **2008**, *41*, 7787. (b) Wan, D. C.; Wang, G. C.; Pu, H. T.; Jin, M. *Macromolecules* **2009**, *42*, 6448. (c) Arumugam, S.; Vutukuri, D. R.; Thayumanavan, S.; Ramamurthy, V. *J. Am. Chem. Soc.* **2005**, *127*, 13200. (d) Baars, M.; Froehling, P. E.; Meijer, E. W. *Chem. Commun.* **1997**, 1959.

(10) (a) Aathimanikandan, S. V.; Sandanaraj, B. S.; Arges, C. G.; Bardeen, C. J.; Thayumanavan, S. *Org. Lett.* **2005**, *7*, 2809. (b) Jansen, J.; Debrabandervandenberg, E. M. M.; Meijer, E. W. *Science* **1994**, *266*, 1226. (c) Jansen, J.; Meijer, E. W.; Debrabandervandenberg, E. M. M. *J. Am. Chem. Soc.* **1995**, *117*, 4417.

(11) (a) Sheiko, S. S.; Sumerlin, B. S.; Matyjaszewski, K. *Prog. Polym. Sci.* **2008**, *33*, 759. (b) Zhang, M. F.; Muller, A. H. E. *J. Polym. Sci., Polym. Chem.* **2005**, *43*, 3461. (c) Beers, K. L.; Gaynor, S. G.; Matyjaszewski, K.; Sheiko, S. S.; Moller, M. *Macromolecules* **1998**, *31*, 9413. (d) Wintermantel, M.; Gerle, M.; Fischer, K.; Schmidt, M.; Wataoka, I.; Urakawa, H.; Kajiwara, K.; Tsukahara, Y. *Macromolecules* **1996**, *29*, 978.

(12) (a) Huang, K.; Canterbury, D. P.; Rzayev, J. *Chem. Commun.* **2010**, *46*, 6326. (b) Huang, K.; Canterbury, D. P.; Rzayev, J. *Macromolecules* **2010**, *43*, 6632. (c) Huang, K.; Rzayev, J. *J. Am. Chem. Soc.* **2009**, *131*, 6880. (d) Mullner, M.; Yuan, J. Y.; Weiss, S.; Walther, A.; Fortsch, M.; Drechsler, M.; Muller, A. H. E. *J. Am. Chem. Soc.* **2010**, *132*, 16587.

(13) (a) O'Reilly, R. K.; Hawker, C. J.; Wooley, K. L. *Chem. Soc. Rev.* **2006**, *35*, 1068. (b) Wooley, K. L. *J. Polym. Sci., Part A; Polym. Chem.* **2000**, *38*, 1397.

(14) Huang, K.; Jacobs, A.; Rzayev, J. *Biomacromolecules* **2011**, *12*, 2327.

(15) Astruc, D.; Boisselier, E.; Ornelas, C. *Chem. Rev.* **2010**, *110*, 1857.