Vesicular self-assembly of comb–dendritic block copolymers†

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New amphiphilic comb–dendritic block copolymers were developed as building blocks that self-assemble into stable vesicular structures with narrow size distribution.

The key to self-assembly lies in the design and synthesis of molecules or macromolecules that organize themselves into desired patterns and functions.[1] Through manipulation of intermolecular noncovalent interactions, block copolymers can self-assemble into a large variety of nano/micro-scaled structures with versatile potential applications.[2,3] Along with chemical composition and functionality, molecular or supramolecular architecture is a key factor that affects the resulting self-assembled structures.[4–8] Among the numerous reported architectures of block copolymers, the introduction of a dendritic structure as a building block for block copolymers provides an attractive set of opportunities.[9–11] The resulting hybrid linear–dendritic block copolymers incorporate the advantages of high functional density from the dendritic architecture and the phase segregated morphological behavior of traditional block copolymers.

Recent work on the self-assembly of linear–linear block copolymer systems has illustrated the ability to form block copolymer vesicles;[7,12–23] compared to traditional/natural phospholipids, polymer vesicles not only have the advantage of superior stability and toughness, but in addition offer numerous possibilities to tailor physical, chemical, and biological properties by variation of block lengths, chemical structure, and conjugation with biomolecules, as well as encapsulant retention. The formation of vesicles relies on the fact that the building blocks (polymer amphiphiles) energetically prefer a bilayer molecular arrangement.

In this communication, we report an amphiphilic biocompatible comb–dendritic block copolymer (**1**) that can self-assemble into bilayer vesicles with narrow size distributions compared to traditional lipid molecules (Fig. 1). This system uses design concepts to yield highly stable submicron- or nano-scale vesicles that contain a functional dendritic exterior and a hydrophobic lipid-like membrane interior. The structure of macromolecule **1** consists of poly(γ-n-dodecyl-L-glutamate) as a hydrophobic comb-like block and a polyester dendron with hydroxyl end groups as a hydrophilic/polar dendritic block. The α-helical conformation of the short poly(γ-n-dodecyl-L-glutamate) imparts a rod-like character to the hydrophobic block, which, when coupled with the presence of an aliphatic side-chain emanating from each repeat unit, creates an unusually hydrophobic comb-rod.[24] The assembly of the comb-like structure generates a basic architecture similar to that of lipid bilayers in which the alkyl side-chains should be able to undergo strong hydrophobic interactions, as illustrated schematically in Fig. 1C. The stiff rod structure and comb side groups of the glutamate block should lower the lateral or in-plane diffusivity of these molecules (*i.e.* convection and diffusion) and thereby further impede morphological destabilization of the polymer vesicles. Additionally, the free hydroxyl groups at the end of dendron segments provide the possibility for further chemical surface modification. Finally, all the building blocks chosen for macromolecule **1** are either biodegradable or biocompatible due to the amino acid backbone and polyester dendron compositions, making them of interest for a range of biomedical applications, *e.g.* drug delivery or imaging.[25–28]

The synthesis of macromolecule **1** consists of three simple steps: 1) preparation of a 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) based polyester dendron with a primary amine group at the root *via* an amidation reaction by using 1,1′-carbonyldiimidazole (CDI), 2) ring-opening polymerization of the *N*-carboxyanhydride (NCA) of γ-n-dodecyl-L-glutamate initiated with the dendritic molecule from step 1, and 3) deprotection of the acetonide protective groups at the periphery of the dendron through transetherification with methanol under acidic conditions. This synthetic strategy provides opportunities towards tunable macromolecular architectures by changing the size of each individual

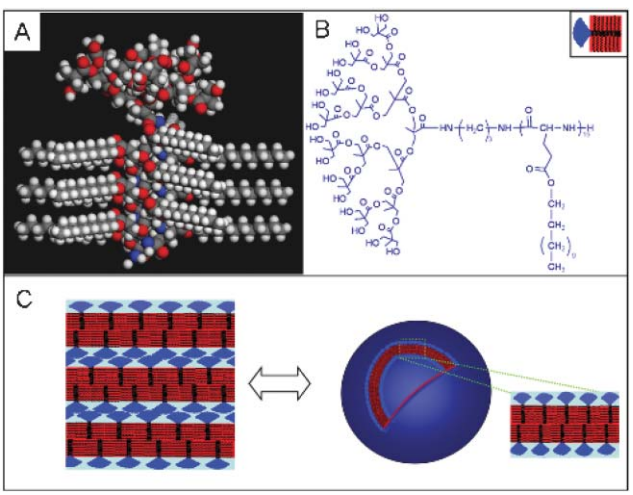

Fig. 1 (A) A space filling molecular model of macromolecule **1**. The three-dimensional image was obtained by modeling with Materials Studio™. Gray atoms: carbon; white atoms: hydrogen; red atoms: carbon; blue atoms: nitrogen. (B) Chemical structure of macromolecule **1**. (C) A cartoon illustration of self-assembly of comb–dendritic block copolymers to form bilayer vesicles. Alkyl chains are in the molten state at room temperature, and the cartoon is not meant to imply ordered alkyl structure.

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block, *i.e.* the dendrimer generation and the polymerization degree of poly(γ-n-dodecyl-L-glutamate). (Please see supporting information for the synthetic details).

Regarding the self-assembly behavior in solution, for amphiphilic linear block copolymers the average molecular shape is based on the relative chain lengths and the radius of gyration of each block in solution, and the equilibrium state chain stretching in the micellar state. The geometrical shape of macromolecule **1** is restricted, in contrast to typically fluctuating, flexible linear polymer analogues, due to the shape persistency of its dendritic block and comb-rod block. Given the estimated size of the dendron head group and length of the rod block, the architecture of macromolecule **1** favors the formation of bilayers according to the theory of Israelachvili *et al.*;[29] *i.e.* the critical packing parameter v/a_0l_c has a value close to one (v, hydrocarbon volume; a_0, optimal headgroup area; l_c, critical chain length). As shown in Fig. 2A, the small angle X-ray scattering (SAXS) measurement of a cast film of concentrated macromolecule **1** solution in THF indicates the scattering characteristic of a lamellar morphology in the solid state with a 1 : 2 : 3 ratio of q values indicating higher order reflections. The observed d-spacing value of 6.67 nm is on the scale of the thickness of a bilayer structure (as shown in the insert cartoon illustration of Fig. 2), which is about 6.3 nm from molecular modeling. Within each bilayer structure, the alkyl side-chains of the comb blocks form paraffin-like crystals at low temperatures,[30] and exhibit a melting transition at −16.2 °C in DSC thermograms (Fig. 2B); thus, at room temperature the alkyl side-chains are in the "molten state". Unlike the homopolymer of poly(γ-n-dodecyl-L-glutamate), the presence of the polar dendritic block hinders the regular packing of the glutamate α-helical backbones, and there is no apparent first-order transition of a thermotropic liquid crystalline phase observed for the material in the bulk state.

When introduced into polar solvents (such as THF and water), macromolecule **1** self-assembles into vesicular structures (Fig. 3) based on a bilayer structure with hydroxyl dendritic blocks at the membrane periphery and the flexible alkyl side-chains sequestered within the vesicle membrane along the rigid α-helix rod. The stability of the bilayer structures is quite pronounced, allowing us to image these structures as spherical polymer vesicles on surfaces using transmission electron microscopy (TEM) without any further stabilization. The samples for TEM measurements are

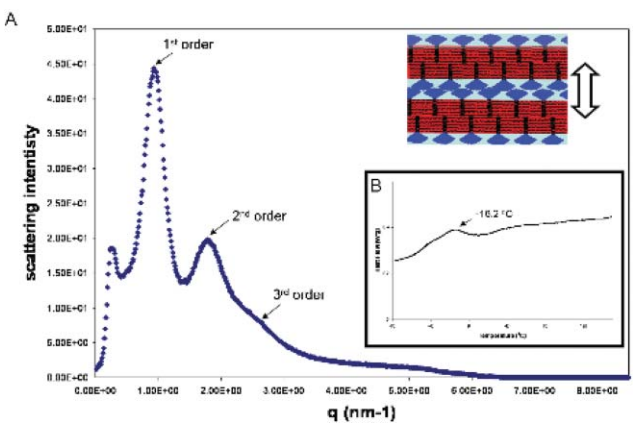

Fig. 2 (A) SAXS of the cast film of macromolecule **1**. (B) DSC thermogram of cast film of macromolecule **1** on heating.

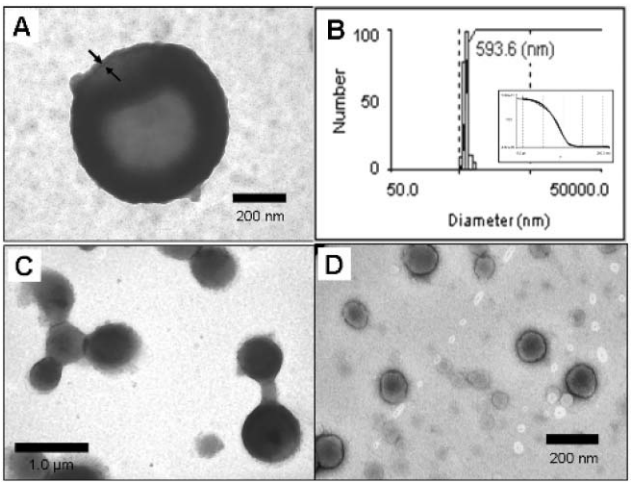

Fig. 3 A) TEM image of a representative polymer vesicle from macromolecule **1** in THF (1.0 mg/ml). B) A representative DLS histogram of polymer vesicles in THF (1.0 mg/ml) (inserts show the autocorrelation functions). C) The membrane fusion/fission between polymer vesicles at a higher concentration in THF (10 mg/ml). D) Polymer vesicles in water (0.1 mg/ml).

prepared by directly dip-casting polymer solutions onto the TEM grids and staining with phosphotungstic acid (1.0 wt% in water). Fig. 3A shows an example of TEM images of these spherical vesicles prepared from THF solution at 1.0 mg/ml. As the arrows indicate, the vesicular wall is about 10 nm in thickness, not inconsistent with SAXS data of the bilayer spacing. From dynamic light scattering (DLS) studies of the same polymer solution (Fig. 3B), the averaged particle size of these vesicles is 600 nm, consistent with TEM observations. The averaged relative standard deviation of the DLS histograms is 4.2%. Such narrow distributions in vesicle size are achieved here without any special preparation techniques (such as liposome-type extrusion methods). This behavior is probably due to the high shape persistence and low polydispersity of each polymer building block. The steric restrictions presented by the dendritic-rod polymer system lead to a limited range of aggregation number and numbers of potential equilibrium structures and sizes, thus yielding unusually low polydispersities in vesicle phases. Interestingly, at a higher concentration (10 mg/mL in THF), the vesicles show fusion/fission type behavior as illustrated in Fig. 3C. Here the fluidity of the vesicle membrane and the resemblance of the assembly to biological processes involving cell membranes are illustrated. Additionally, the polymer vesicles can be redistributed into water from organic solvents such as THF. The resulting vesicles in water have a much smaller size of approximately 100 nm in diameter (Fig. 3D). Such size ranges are relevant for cellular uptake, and present the possibility of utilizing these polymer vesicles as long-circulation time liposome-type drug delivery vesicles with further surface functionalization (*i.e.* PEGylation and targeting ligand modification).[31]

Regarding the potential biomedical applications for vesicular encapsulation and delivery therapeutic reagents, the biocompatibility of macromolecule **1** was evaluated *via* cytotoxicity studies *in vitro* with Hep G2 human hepatocellular carcinoma cells. Based on cell viability relative to the control (no polymer added), the cytotoxicity of macromolecule **1** was negligible at 24 h of

incubation for all polymer test concentrations (10^{-4} to 10^{-1} mg/ml). Even at 48 h of incubation, macromolecule **1** showed no significant cytotoxicity at concentrations up to 0.1 mg/ml, which is its solubility limit in water.

In summary, we report a new amphiphilic biocompatible comb–dendritic block copolymer that can self-assemble into bilayer vesicles by mimicking membrane lipids. Constructed through biodegradable amide and ester bonds, macromolecule **1** has a well-defined structure and molecular shape persistency based on a poly(γ-n-dodecyl-L-glutamate) rod-comb block and a hydroxyl–terminated polyester dendritic block. The assembled vesicles have high stability and defined particle sizes which can be tuned by changing solvent quality without the need for additional process techniques. In addition, the increased functionality of self-assembling block copolymers is a desired feature of emerging polymer vesicular systems for potential applications in drug/gene delivery and biosensors. The reported polymer vesicles possess a dendritic hydroxyl surface, and many functions can be implemented through chemical modifications. The preparation of such functionalized vesicles from comb–dendritic block copolymers (*e.g.* with biospecific ligands to improve biologically relevant affinity enhancement) is currently in progress.

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