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Organic Nanospheres with Internal Bicontinuous Structure and Their Responsive Phase Inversion

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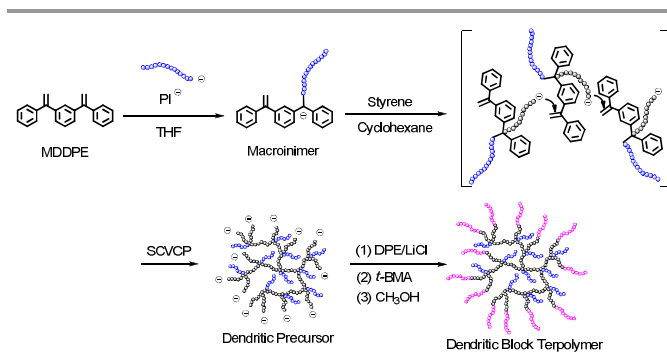
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Nanospheres with internal bicontinuous structure were obtained through hierarchical self-assembly of dendritic block terpolymer in selective solvents. The self-assembly underwent a unique three stage process involving unimolecular micelle formation, multimicelle aggregation and microphase separation within self-confined space. Reversible phase inversion of the nanospheres in response to the solvent environment was observed.

Self-assembly is an essential rule in life process for the formation of hierarchical structures, and is becoming a powerful tool for bottom-up construction of ordered nanomaterials.^{1–2} Block copolymer in solution is a widely studied model system in which the copolymer self-assembles into a variety of morphologies, such as spherical micelles, vesicles, cylindrical, fibril, multicompartiment and other complex micelles, etc.^{3–8} The morphology formation is determined by both thermodynamic and kinetic factors which can be subsequently translated into molecular parameters of the polymers. One of the most important factors is the molecular architecture that may influence volume ratio and geometric sequence of solvophobic and solvophilic domains.⁹ Particularly, hyperbranched polymers self-assembled into macroscopic tubes, large complex micelles (LCMs) and large compound vesicles (LCVs).^{10–12} These featured morphologies are formed by a unique process involving multimicelle aggregates (MMA), which obviously benefits from the fact that hyperbranched or dendritic polymers behave as unimolecular micelles in selective solvent.^{11,12} LCM may be thermodynamically metastable and can serve as a template that allows further evolution of morphology inside its confined space.¹³ The morphology formation in a confined space is different from those in bulk due to geometrical restriction and possible selective adsorption of one phase to the interface.¹⁴ This will in turn give inspiration, and challenge, for molecular design of dendritically branched polymers.

In this work, we report fabrication of structured nanospheres from the self-assembly of an ABC-type dendritic terpolymer containing

polyisoprene (PI), polystyrene (PS) and poly(*tert*-butyl methacrylate) (PtBMA) segments. The terpolymer is synthesized from self-condensing vinyl copolymerization based on an anionic inimer developed in our laboratory. As shown in Scheme 1, the inimer is a monoadduct of polyisoprenyllithium and 1,3-bis(1-phenylethenyl)benzene (MDDPE), possessing a PI chain, a double bond and a living anion, made from quantitative monoaddition in THF solvent. It is coded hereinafter macroinimer due to the presence of the PI segment. The macroinimer is not able to homopolymerize but can copolymerize with styrene to form dendritic copolymers.¹³ Reinitiation of *tert*-butyl methacrylate (*t*-BMA) results in the formation of dendritic terpolymers Dendri(PI-*b*-PS-*b*-PtBMA) (Figure S2 in the Supplementary Information). In the structure of the terpolymer, individual PI and PtBMA segments are isolated and connected to a dendritic PS skeleton. The composition is determined by NMR and listed in Table S1 in the Supplementary Information, which is close to the feed ratio of monomers.



Scheme 1. Synthesis of dendritic block terpolymer through anionic self-condensing vinyl copolymerisation (SCVCP) of polyisoprene macroinimer, styrene and *tert*-butyl methacrylate.

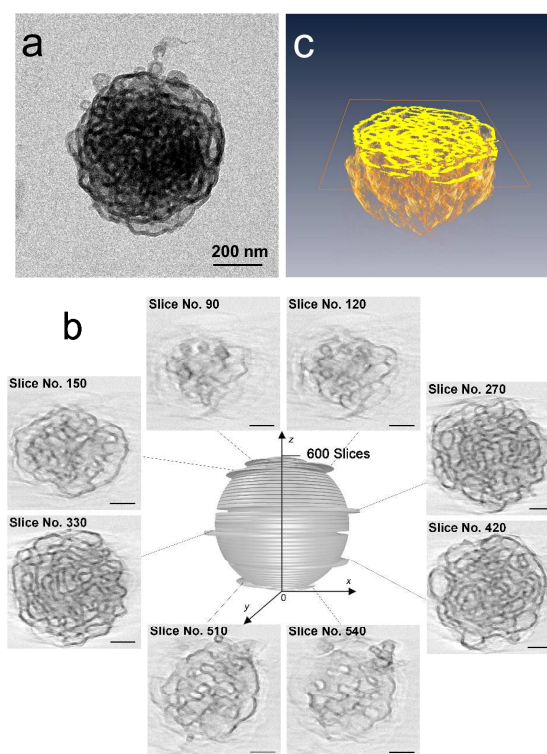


Figure 1. CryoTEM results of self-assembly of Dendri(PI₁₄-*b*-PS₆₄-*b*-PtBMA₂₂) in ethanol/THF (10/1, v/v) mixture. (a) cryoTEM image of a nanosphere formed from self-assembly of the terpolymer; (b) Tomographic slices of different cross-sections along z-axis (scale bars 200 nm); (c) 3D reconstruction of a segmented volume of the nanosphere.

We investigate the self-assembly of a dendritic product, Dendri(PI₁₄-*b*-PS₆₄-*b*-PtBMA₂₂) ($M_{n,GPC} = 26\ 000\ \text{g/mol}$), where the subscripts represent the weight percent of the corresponding segment, by means of selective solvent titration and TEM observation. First, the terpolymer is dissolved in THF to form a transparent solution (1.0 mg/mL). Then ethanol is titrated to the solution and blue opalescence appears. Ethanol is a good solvent for PtBMA but non-solvent for both PS and PI blocks. Further addition of ethanol up to ten-fold of THF (volume ratio) leads to the formation of a stable suspension. CryoTEM shows that large sphere-like particle with a rather hollow network-like internal structure exists in the suspension (radius ca. 300 nm) (Figure 1a). Conventional TEM gives similar results (Figure S3a in the Supplementary Information). Close analysis of tomographic slices of cryoTEM morphology reveals that the network is composed of interconnected curly wires (Figure 1b). The shape of the wires is confirmed by 3D reconstruction of a segmented volume of the aggregate shown in Figure 1c. The space between the wires, as indicated by low contrast regions, is filled with mixed solvent, ethanol/THF. This is also implied by the porous structure in SEM observation (Figure S3b in the Supplementary Information). Thus the nanosphere possesses bicontinuous morphology through interior microphase separation. The formation of bicontinuous structures in confined space were theoretically predicted and have been observed in assemblies of linear and comb-like copolymers.^{15–19} To the best of our knowledge, this is the first report of such structure obtained from dendritic copolymer.

In order to investigate the mechanism of self-assembly in the particles, we traced the morphology evolution by conventional TEM during ethanol titration process. This was done by addition of different amounts of ethanol to four parallel homogenous THF solutions of the dendritic terpolymer. At the volume ratio of ethanol/THF = 1/1 (v/v), the solution is transparent but the TEM result shows small aggregates (Figure 2a). When the volume ratio reaches to ethanol/THF = 2/1, the solution becomes opaque and TEM shows large size aggregates (diameters ca. 500–800 nm) with rugged outer edge (Figure 2b). Further addition of ethanol causes phase separation at the periphery of the aggregates (Figure 2c). Phase separation develops from peripheral to the inner part of the nanosphere along with continuous addition of ethanol. The formation of well-developed bicontinuous structure is nearly complete at ethanol/THF = 4/1 (v/v) (Figure 2d).

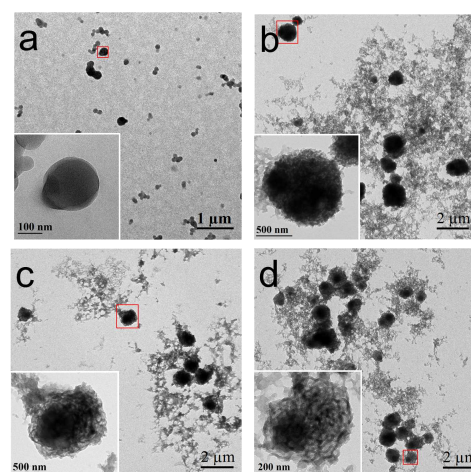


Figure 2. Conventional TEM images monitoring the process of self-assembly at different ethanol/THF volume ratios (original concentration 1.0 mg/mL): (a) 1/1; (b) 2/1; (c) 3/1; (d) 4/1.

Dynamic light scattering (DLS) on the solution provides more direct evidence of the self-assembly. As shown in Figure 3, the dendritic terpolymer in THF shows hydrodynamic radius (R_h) of 9 nm. Upon addition of ethanol (~20% of THF volume), R_h reduced to 5 nm. Further addition of ethanol (1 fold in volume) led to a bimodal distribution in DLS profile, with peak R_h ~5 and 80 nm, respectively. Further titration of ethanol resulted in intensity decrease, and finally disappearance, of the peak at lower R_h , along with the increase of peak at larger R_h .

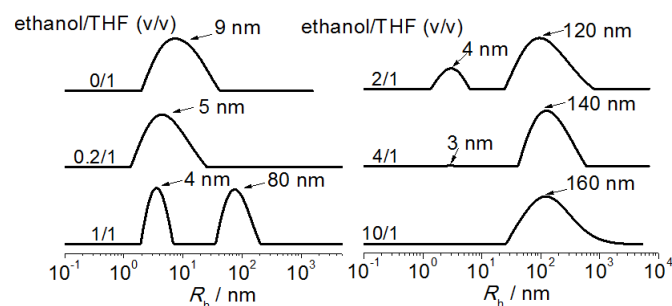


Figure 3. DLS results on solutions of dendritic terpolymer in mixed solvents of various volume ratios.

We propose that the self-assembly undergoes a three-stage process. First, unimolecular micelles are formed through molecular conformation rearrangement when ethanol is added to THF solution. Solvophilic PtBMA segments form the shell while solvophobic dendritic PS with attaching PI segments form the core. This is indicated by the decrease in R_h upon addition of ethanol. Second, addition of more ethanol leads to the formation of LCM through the aggregation of unimolecular micelles. The aggregation is driven by lowering the interfacial energy between the core and the solvent. The mechanism of MMA process is supported by the rugged instead of smooth outmost layers and loosely packed bulk of the particles. In a single sphere, PtBMA forms the solvated continuous phase that spans from the surface layer to the penetrating inner part. The loose packing of the polymer substances in the particle allows double diffusion of solvents, i.e., THF moving out while ethanol moving in. Third, the drift in volume fraction of the solvent drives the interior of the sphere to self-assemble into a bicontinuous morphology. The network of polymer phase has a structure of interconnected cylindrical micelles with PtBMA shell and PS/PI core. The thickness of the cylinder is estimated to be 13 ± 1 nm in Figure 1b, which is close to double R_h of the dendritic block terpolymer, 18 nm. This indicates that the formation of the cylindrical micelles is achieved through molecular stacking of the dendritic terpolymers due to aggregation of the dendritic core.

Phase separation between PS and PI segments inside the particle was not observed. TEM images of selective stained samples using OsO_4 or RuO_4 show uniformly enhanced contrast (Figure S4 in the Supplementary Information). This can be a consequence of three reasons: i) the fraction of PI is low ($\text{PI/PS} = 1/4$, w/w); ii) the dendritic structure prevents an efficient phase separation; iii) the selectivity of the solvents for PS and PI is not high. Nevertheless, the presence of PI segment is important to ensure the mobility of solvophobic PS segments due to its low glass temperature. Indeed, replacing PI by PS segments in the synthesis starting from a PS macroinimer, instead of a PI macroinimer, leads to the formation of smaller sized micelles without fine structure (Figure S6a in the Supplementary Information).

The dendritic structure plays a key role in the formation of bicontinuous morphology. These highly branched polymers not only form unimolecular micelles that tend to aggregate into LCM, but also facilitate the phase separation inside the nanosphere. In a control experiment, a linear block terpolymer, $\text{PI}_{13}\text{-}b\text{-PS}_{66}\text{-}b\text{-PtBMA}_{22}$, prepared from sequential anionic polymerization with similar composition, self-assembles into large size micelles without phase separated inner structure. It was recently reported that a diblock copolymer, poly(ethylene oxide)-*b*-poly(*n*-butyl methacrylate), self-assembled into bicontinuous structure when the cosolvent were carefully adjusted.¹⁹ Thus the present results and those in literature illustrate that both the architecture and the composition, among others, determine the phase diagram because both of them affect the packing parameter.

Furthermore, it seems that the weight ratio of PtBMA to the sum of PS and PI (78/22, w/w) falls into the region of cylindrical micelle of the phase diagram. Increasing the length of PtBMA chains results in different self-assembly behaviour. In TEM image of self-assembly of another product in Table S1, $\text{Dendri}(\text{PI}_6\text{-}b\text{-PS}_{28}\text{-}b\text{-PtBMA}_{66})$, only unimolecular micelles and large size vesicles are observed (Figure S6b

in the Supplementary Information). The aggregation tendency of the unimolecular micelles to form LCMs is alleviated by stronger shielding effect of longer PtBMA chains. Therefore, these aggregates cannot develop into bicontinuous micelles.

Interestingly, the structured nanosphere undergoes reversible phase inversion in response to solution environment. Addition of *n*-heptane (ten-fold in volume), a good solvent for PI but nonsolvent for PS and PtBMA, to the dispersion of bicontinuous aggregates in ethanol/THF results in reversal of the morphology. PI segments are extracted from inner phase to outer solvophilic part, forming "jellyfish" like morphology owing to vitrification of PS domains to keep the integrity of the particle (Figure 4b). The outstretched PI segment can be reversibly taken back into the particle, upon switching the solvent to ethanol/*n*-heptane (10/1 v/v), to recover the bicontinuous structure (Figure 4c). In this reverse process, it is necessary to add a small amount of THF (1/10 of *n*-heptane) to swollen the PS and PtBMA domains, therefore increasing the mobility of polymer segments. Without the aid of THF, we have only observed large size particles with homogenous but no bicontinuous phase by TEM (Figure S9 in the Supplementary Information). Note that the particle number in Figure 4b and c are clearly smaller than that in Figure 4a. This is simply due to the dilution effect of the solvent addition.

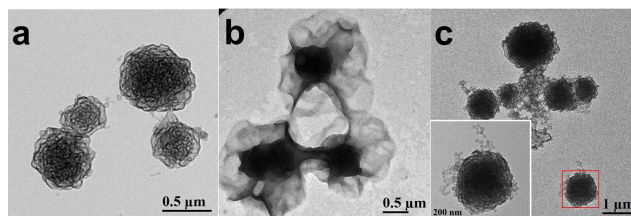


Figure 4. Conventional TEM images demonstrating reversible phase inversion of the nanospheres in different solvents. (a) nanospheres with bicontinuous structure in ethanol/THF (10/1); (b) "jellyfish" like aggregates formed upon addition of *n*-heptane (ten-fold in volume); (c) recovery of the bicontinuous structure upon addition of ethanol (ten-fold in volume) to the dispersion of "jellyfish" like aggregates.

To summarize, we have observed that a dendritic block terpolymer, $\text{Dendri}(\text{PI}_{14}\text{-}b\text{-PS}_{64}\text{-}b\text{-PtBMA}_{22})$, self-assembles into nanospheres with interior bicontinuous structure. The mechanism of the assembly is basically a phase separation process within self-confined space. This is obviously a consequence of the dendritic structure that tends to form unimolecular micelle and subsequently LCM. The nanospheres undergo reversible phase inversion in response to the solvent change. Therefore, the present work may provide a new prototype process for the preparation of responsive organic nanoparticles with well-defined internal structures.

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Notes and references

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