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“Shish-Kebab” Supermicelles from the Hierarchical Assembly of Cylindrical Block Comicles Mediated by Spatially Confined Hydrogen-Bonding Interactions

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ABSTRACT: Hydrogen (H)-bonds are among the most common interactions used by nature for the creation of hierarchical structures from smaller building blocks. Herein, we describe an in-depth study of the hierarchical assembly of cylindrical block comicles with a crystallizable poly(ferrocenyldimethylsilane) (PFS) core via H-bonding interactions into complex supermicellar structures. Well-defined block comicles bearing H-bond donor (H,D) segments (M(PFS-b-PMVSOH)), or H-bond acceptor (H,A) segments (M(PFS-b-P2VP)), and non-interacting (N) segments (M(PFS-b-PtBA)) were created by the living crystallization-driven self-assembly (CDSA) method (PMVSOH = hydroxyl-functionalized poly(methylvinylsiloxane), P2VP = poly(2-vinyl pyridine) and PtBA = poly(tert-butyl acrylate), M = micelle segment). Due to the control provided by the living CDSA approach, both the block comicles and the individual segments were monodisperse in length, which facilitated their predictable hierarchical assembly into higher-level structures. Two cases were investigated in detail: firstly, the interaction of N-H,D-N triblock comicles and the H,D homopolymer PMVSOH, and secondly, N-H,A-N triblock comicles with very short H,A cylinders (seeds). By manipulation of several factors, namely coronal steric effects (via the PtBA corona chain), and attractive interaction strength (via the H-bonding interaction between P2VP and PMVSOH), the aggregation of the triblock comicles could be controlled, and well-defined multimicron-size structures such as “shish-kebab”-shaped supermicelles were prepared. The ability of the seeds adsorbed on the block comicles to function as initiators for living CDSA to generate fence-like “shish-kebab” superstructures was also explored.

Nature’s biological diversity relies on multitier self-assembly based on weak non-covalent interactions such as electrostatic, hydrogen (H)-bonding, and hydrophobic forces. A classic example is provided by the interstrand H-bonds that stabilize the double helical structure of DNA, an essential element for genetics. Furthermore, for polypeptides, the precise positioning of the H-bonding segments is of crucial importance for the correct folding of polypeptide chains and thus the function of proteins. Inspired by Nature, scientists have utilized the H-bonding interaction to direct the assembly of artificial materials into higher-level structures. For example, the specific H-bond interactions between the DNA base-pairs have been used to direct the assembly of synthetic DNA molecules into highly regulated and exquisitely controlled architectures (DNA origami). In synthetic materials, H-bonding interactions between small molecules, polymer/small molecule pairs, and polymer/polymer pairs have been extensively exploited to create higher-level structures. Self-assembly of block copolymers (BCPs) is an efficient bottom-up approach to achieve nanoscopic structures with complex architectures and multiple functionalities. However, only a few examples have been reported on the hierarchical self-assembly of BCP micelles to form supermicellar micelle-scale structures and these have involved the utilization of electrostatic or amphiphilic interactions. The use of H-bond donor-acceptor binding is an appealing approach but difficulties are anticipated concerning tuning of the strength of the interaction and the precise positioning the interacting segments to produce well-defined supermicelles. Moreover, to avoid the build-up of defects from the formation of undesirable kinetically-trapped structures, hierarchical assembly requires robust building blocks of near-identical size and periodicity and some degree of dynamic character to the interactions. In cases where the structures can be precisely controlled, spherical Janus and patchy particles based on BCPs have been shown to be very useful building blocks.

Among the cases of hierarchical assembly of BCP micelles, the case of of cylinders is particularly interesting. This can resemble the assembly of collagen fibrils to form the main scaffold in bone. For example, a synthetic collagen mimic polypeptide has been shown to assemble into nanofibers, which can further hierarchically assemble to form hydrogels. Similarly, Janus cylinders made from synthetic BCPs can aggregate through well-defined solvophobic regions in selective solvents to form cylindrical bundles. Composite cylindrical structures have been formed by mixing two oppositely charged cylinders or cylinders and spheres, and the generation of regular hierarchical structures from amphiphilic cylindrical micelles has recently been achieved.
We have previously shown that BCPs with a crystallizable poly(ferrocyenylidimethylsilane) (PFS) core-forming block can self-assemble into cylindrical micelles in selective solvents for the corona-forming block. Significantly, the termini of the crystalline PFS micelle core remain active to further growth via a process termed living crystallization-driven self-assembly (CDSA). Following sonication to form short seeds, near monodisperse cylinders ($L_w/L_n < 1.1$, $L_w$ and $L_n$ are weight- and number-average lengths, respectively) with controlled lengths from ca. 40 nm – 2 μm have been obtained using this living CDSA approach by variation of the unimer-to-seed ratio. It is also possible to generate complex micelle architectures with segmented coronas including multiblock comicelles such as monochrome and multicolor fluorescent “barcode”, multi-armed micelles and non-centrosymmetric structures. The living CDSA process has also been extended to BCPs containing other crystallizable core-forming blocks, including polylactide, poly(2-vinyl pyridine), and a non-interacting (N) segment forming BCPs via H-bond interactions. The work demonstrates the creation of complex supermicelle architectures from building blocks created by living CDSA. In particular, we focus on the interaction of the block comicelles with homopolymers or short seeds via H-bonds to yield composite supermicelles with “shish-kebab” architectures.

Results

For the systematic study of the triblock comicelle assembly, three kinds of BCPs were used (Scheme 1): a H-bond donor (H$_A$) segment-forming BCP PFS$_{280}$-b-PMVSOH (PMVSOH = hydroxyl-functionalized poly(vinylmethylsiloxane)), a H-bond acceptor (H$_A$) segment-forming BCP PFS$_{280}$-b-P2VP (P2VP = poly(2-vinyl pyridine)), and a non-interacting (N) segment forming BCP PFS$_{280}$-b-PtBA (PtBA = poly(2-ethyl butyl acrylate)). All BCPs were prepared by sequential living anionic polymerization, and all contain a crystallizable PFS core-forming block. Also used was an H$_B$ homopolymer PMVSOH, which was obtained via functionalization of PMVS by thiol-ene click chemistry. The characterization of all the polymers is included in Table S1.

For convenience, as the crystalline PFS core was a common feature, all of the triblock comicelles are depicted in an abbreviated form that reflects their coronal chemistry (for example, triblock comicelle M(PFS$_{280}$-b-PtBA$_{280}$)$\_b$-M(PFS$_{280}$-b-P2VP$_{448}$)$\_b$-M with a central M(PFS$_{280}$-b-P2VP$_{448}$) segment of 50 nm in length, is described as N$_{280}$H$_A$$_{480}$ (50 nm)-N$_{280}$. The subscript numbers represent the degree of polymerization (DP) of the corona-forming block. The length in the brackets is the length of the central (in this case...
H$_2$) segment. For H$_2$ segments used in the current study, since only one diblock copolymer, PFS$_{280}$-b-PMVSOH$_{120}$ is used, the H$_2$ segments are all described as H$_2$1,3,12 segment. As we discussed previously, the length of soluble corona chains (or corona layer thickness) of PtBA or P2VP is greatly dependent on the DP of PtBA or P2VP blocks, respectively. Thus, herein we will use DP$_{PtBA}$ or DP$_{P2VP}$ as an indication of the length of the corona PtBA or P2VP chains, respectively.

The triblock comicles, for example N-H$_2$-N cylinders, were prepared by seeded growth from short H$_2$ cylindrical micelles (seeds) via living CDSA (Scheme 1(I)). Firstly, low dispersity H$_2$ micelle seeds were prepared by the addition of PFS$_{280}$-b-PMVSOH$_{120}$ unimers in THF to a solution of small PFS$_{280}$-b-PMVSOH$_{120}$ crystallites in isopropanol (i-PrOH) (ca. 40 nm in length, prepared by sonication of long cylindrical micelle precursors). The length of the H$_2$ micelle seeds was controlled by the ratio of the PFS$_{280}$-b-PMVSOH$_{120}$ unimers to the small crystallites (see Table S2). To the i-PrOH solution of the H$_2$ seeds, the desired amount of PFS-b-PtBA unimers in a small portion of THF was added to achieve the desired N-H$_2$-N cylinder length. N-H$_2$-N triblock comicles were prepared in an analogous manner from H$_2$ seeds. All the seed cylinders and triblock comicles were characterized by transmission electron microscopy (TEM). As can be appreciated from the TEM images and summarized data (Table S2-S3, Figure S1-S4), both seeds and triblock comicles were uniform in length, due to the control arising from the living CDSA method. This ensured that all the micelles from the sample possessed very similar structure, a feature likely to facilitate their controlled hierarchical assembly.

Two approaches to the hierarchical assembly of the cylindrical block comicles have been explored in this study. These are illustrated in Scheme 1 (II). The first involves the assembly of triblock comicles (N-H$_2$-N) through H-bonding with H$_2$ homopolymer (PMVSOH), and the second, triblock comicles (N-H$_2$-N) with short cylindrical seeds (H$_2$).

1. Interactions between H$_2$ homopolymer and H$_A$ short cylinder seeds: formation of precipitates

Over the past few decades, H-bonding interactions have been widely used to prepare non-covalently linked BCPs, for layer-by-layer assembly, and to induce the micellization of BCPs. However, if no spatial confinement is introduced, the hierarchical assembly of BCP micelles via H-bonding usually leads to precipitates in solution due to the abundant complementary interactive sites among different chains to form “H-bond networks.”

Similarly, when H$_2$ homopolymer PMVSOH$_{105}$ and H$_A$$_{448}$ seeds (ca. 50 nm) were mixed in i-PrOH (a selective solvent for PtBA, PMVSOH and P2VP blocks), precipitates were observed in 30 s (hydroxyl / pyridyl mole ratio = 1 / 1) and large aggregates could be observed by TEM (Figure S5(c)). Meanwhile, at other mole ratios, the solutions turned cloudy and small aggregates were observed by TEM (Figure S5).

2. Interactions between H$_2$ homopolymer and triblock comicle N-H$_A$-N: the formation of “shish-kebab” structures

Based on these observations, next we investigated the more controlled interactions between the H$_2$ homopolymer and N-H$_A$-N triblock comicles, in which the H$_2$ polymer chains were confined to the corona of the central segment of the cylindrical micelle building block (Figure 1 (c)). The N segments are non-interactive with other segments and were anticipated to act as shielding segments to increase the colloidal stability of the resulting supermicellar structures. Indeed, TEM analysis showed that addition of H$_2$ homopolymer to the i-PrOH solution of the triblock comicle N$_{280}$-H$_{448}$ (50 nm)-N$_{280}$ at a mole ratio of hydroxyl/pyridyl groups of 5 / 1 led to assembly of the comicles through the central H$_2$ coronal segments in a parallel fashion, forming “shish-kebab” supermicelles (Figure 1 (c) and Figure S6(c, d)). The H$_2$ homopolymer chains functioned as a “glue” to enable the assembly of triblock comicles via H-bonding interactions. By varying the mole ratio of hydroxyl / pyridyl groups through the series from 1 / 2 to 1 / 1, 1 / 5, 1 / 10, 1 / 20 and 100 / 1, it was established that the “shish-kebab” supermicelles were formed only at approximately equal hydroxyl / pyridyl ratios. Meanwhile, the use of either a very low or very high mole ratio of hydroxyl / pyridyl groups led to a significant reduction of the aggregation number (or arm distribution) in each supermicelle (Figure 1 and Figure S6). On the other hand, our experimental results suggested that concentration did not have significant effect on the morphology of the supermicelles (see Figure S7).

![Figure 1](image-url)
TEM images of the supermicelle structures formed by H\textsubscript{D} homopolymer PMVSOH\textsubscript{105} with (a-c) N-H\textsubscript{A,448}-N cylinders with DP\textsubscript{PBA} = (a) 600, (b) 460, and (c) 170, and with (d-f) N\textsubscript{280}-H\textsubscript{A}-N\textsubscript{280} cylinders with DP\textsubscript{P2VP} = (d) 250, (e) 448, and (f) 760. Hydroxyl/pyridyl mole ratio = 5/1, final concentration of H\textsubscript{A} segments = 0.02 mg/mL. The aggregation in the case of (c) leads to much more tightly packed comicelles than in the case of (f) which leads to poorer resolution of the individual structures. Scale bars are 1 μm in the images and 200 nm in the insets.

To investigate the steric hindrance effects that arise from the corona chains of the N segments on supermicelle formation, four triblock comicelles N-H\textsubscript{A,448}(50 nm)-N with the same H\textsubscript{A} segments and different N segments with DP\textsubscript{PBA} = 600, 460, 280 and 170 were prepared (from four different N segment-forming PFS-6-PtBA diblock copolymers, see Table S1). The triblock comicelles were mixed with same H\textsubscript{D} homopolymer PMVSOH\textsubscript{105} at hydroxyl/pyridyl mole ratio = 5/1 in i-PrOH.

TEM results showed that when DP\textsubscript{PBA} = 600 (Figure 2(a)), the triblock comicelles either remained as individual entities or only formed “dimers” with two triblock comicelles attached to one another. In contrast, when DP\textsubscript{PBA} = 460 (Figure 2(b)) and 280, “shish-kebab” supermicelles were formed. (Figure 1(c)). Furthermore, when DP\textsubscript{PBA} = 170, the triblock comicelles formed large aggregates with three-dimensional structures (Figure 2(c), additional low-resolution TEM images of the superstructures are included in Figure S8).

Similarly, the strength of the attractive H-bonding interactions could be tuned by using H\textsubscript{A} segment-forming diblock copolymers with different numbers of H-bonding sites. Superstructures were prepared from three different triblock comicelles N\textsubscript{280}-H\textsubscript{A}(50 nm)-N\textsubscript{280} with similar N segments (corona chain length DP\textsubscript{PBA} = 280 and N segment length = 100–200 nm) but different values of DP\textsubscript{P2VP}. Under the same experimental conditions, after the addition of H\textsubscript{D} homopolymer, PMVSOH\textsubscript{105}, the triblock comicelles formed only “oligomers” (aggregates of 1–3 micelles) when DP\textsubscript{P2VP} = 250 (Figure 2(d)), but formed “shish-kebab” supermicelles when DP\textsubscript{P2VP} = 448 (Figure 2(e)). Large three-dimensional aggregates were observed when DP\textsubscript{P2VP} = 760 (Figure 2(f), additional TEM images of the superstructures are included in Figure S9).

Figure 3. TEM images of supermicellar structures prepared by mixing H\textsubscript{D} homopolymer PMVSOH\textsubscript{105} and N\textsubscript{280}-H\textsubscript{A,448}-N\textsubscript{280} with hydroxyl/pyridyl mole ratio = 5/1, final concentration of H\textsubscript{A} segments = 0.02 mg/mL, and length of H\textsubscript{A} segment = (a) 50 nm, (b) 190 nm, and (c) 380 nm. Scale bars are 1 μm and 200 nm in the inset.

An alternative method with which to strengthen the attractive interaction is to increase the length of the H\textsubscript{A} segments. Three different triblock comicelles N\textsubscript{280}-H\textsubscript{A,448}-N\textsubscript{280} with a similar N segment length = 100–200 nm and different lengths...
for the H₄ segment (50 nm, 190 nm and 380 nm) were therefore prepared and studied. Upon addition of H₄ homopolymer, all three samples gave supermicelles with structures based on parallel-packed triblock comicles however key differences were observed (Figure 3). When the H₄ segment was short (50 nm), only “shish-kebab” supermicelles were formed, and the triblock comicles were packed side-by-side and the supermicelles only elongated in one direction (Figure 1(c), 2(e) and 3(a)). However, when the length of the H₄ segment increased to 190 nm, the triblock comicles were not only able to pack in a parallel fashion in one direction but also in three dimensions (Figure 3(b)), forming multilayered bundles. When the length of the H₄ segment was further increased to 380 nm, the size of the supermicelles was greatly increased (Figure 3(c)), and a multilayered bundle structure is clear in the inset image (for additional TEM images see Figure S10).

Figure 4. TEM images of “shish-kebab” superstructures prepared by mixing H₄ homopolymer PMVSOH₂₈₀ with (a) N₂₈₀-H₄₇₆₀ (50 nm)-N₂₈₀ (N segment length = 450 nm), (b) N₁₇₀-H₄₄₈₈ (50 nm)-N₁₇₀ (N segment length = 450 nm), (c) mixtures of N₂₈₀-H₄₄₈₈ (50 nm)-N₂₈₀ triblock comicles with N segment length = 450 nm and 150 nm, hydroxyl / pyridyl mole ratio = 5 / 1, final concentration of H₄ segments = 0.02 mg / mL. Scale bars are 1 μm for the images and 200 nm for the insets. Shown in (d) is the schematic illustration of the superstructures shown in (c).

In contrast, increasing the length of the N segments did not significantly change the morphology of the resultant supermicelles. As shown in Figure 4(a,b) and Figure S11(a-c), three different triblock comicles N₂₈₀-H₄₇₆₀ (50 nm)-N₂₈₀-N₂₈₀-H₄₄₈₈ (50 nm)-N₂₈₀ or N₁₇₀-H₄₄₈₈ (50 nm)-N₁₇₀ with longer N segments (450 nm) were used to replace those used in the previous set of experiments where the length of the N segment was around 100 ~ 200 nm. Similar “shish-kebab” supermicelles were observed. When the two kinds of N₂₈₀-H₄₄₈₈ (50 nm)-N₂₈₀ triblock comicles (length of N segments = 150 nm and 450 nm, respectively) were mixed in i-PrOH, after the addition of H₄ homopolymer, PMVSOH₂₈₀, “shish-kebab” supermicelles were also formed, but with alternating long and short triblock comicles, as shown in Figure 4(c, d) and Figure S11(d).

We also measured the apparent hydrodynamic diameter of the initial triblock comicles N₂₈₀-H₄₄₈₈ (50 nm)-N₂₈₀ (with N segment length = 450 nm) and the corresponding “shish-kebab” supermicelles via dynamic light scattering experiments (DLS) (Figure S12). The apparent hydrodynamic diameter of the triblock comicles N₂₈₀-H₄₄₈₈ (50 nm)-N₂₈₀ (with N segment length = 450 nm) increased from 230 nm to 615 nm after the addition of H₄ homopolymer PMVSOH₂₈₀. This increase in apparent hydrodynamic size is clearly consistent with the formation of “shish-kebab” supermicelles in solution rather than during solvent removal on drying. The result is also consistent with our previous characterization of supermicelle formation in solution using non-covalent interactions by laser scanning confocal fluorescence microscopy (LSCFM).


Figure 5. TEM images (a, b, d), AFM height image (c) and schematic illustrations (e) of composite “shish-kebab” structure from N₂₈₀-H₁₂₀ (600 nm)-N₂₈₀ triblock comicles with (a) H₄₂₅₀ seeds, (b, c) H₄₄₈₈ seeds, and (d) H₄₇₆₀ seeds (all the seeds are around 50 nm, see Table S2 and Figure S2). The hydroxyl / pyridyl mole ratio = 1 / 2. Scale bars are 2 μm, and 200 nm in the inset and AFM images.

Next, we investigated the hierarchical assembly behavior of N-H₄-N triblock comicles and H₄ seeds at various hydroxyl / pyridyl mole ratios (Figure S13). It was revealed by TEM analysis that excess pyridyl groups were necessary (hydroxyl / pyridyl mole ratio = 1 / 2, Figure S13(b)) for discrete composite block comicles to be formed. The resulting structures possessed H₄ seeds attached to the surface of the central H₄ segments, and resembled the composite “shish-kebab” structure. The presence of a small number of free H₄ seeds was
also detected. The yields of each kind of supermicelle in this study are summarized in Table S4.

We then explored the analogous assembly process for N_{280}H_{120}-N_{280} triblock comicelles and H_{A} seeds where the latter were made from different PFS-b-P2VP diblock copolymers. Three kinds of H_{A} seeds, H_{A,250}, H_{A,448} and H_{A,760} with different DP values for the P2VP block but of similar length (ca. 50 nm) were separately added into the i-PrOH solution of N_{280}H_{120}-N_{280} triblock comicelles and the TEM images of the resulting supermicelles are shown in Figure 5. Interestingly, the number of H_{A} seeds that could be adsorbed onto the triblock comicelles varied significantly. With an increasing DP value for the P2VP block in the H_{A} seed, the number of adsorbed seeds decreased, as illustrated in the scheme in Figure 5(e).

![Figure 6](image_url)

Figure 6. TEM images (a, b) and schematic illustration (c) of composite “shish-kebab” supermicellar structures prepared by mixing H_{A,448} seeds (50 nm, L_{w} / L_{a} = 1.1) with N_{280}H_{120}-N_{280} cylinders. The lengths of H_{D} segments are (a) 880 nm, and (b) 3.2 μm, respectively. The mole ratio of hydroxyl / pyridyl = 1 / 2. Scale bars are 1 μm.

A possible explanation for this phenomenon starts from the assumption that increasing the DP value for the P2VP block in the H_{A} seed provides more H-bonding accepting sites. Therefore fewer seeds are needed to bind to the existing H-bond donating sites in the central H_{D} segments of the N_{280}H_{120}-N_{280} triblock comicelles. A steric effect may also play a role and a significant increase in hydrodynamic size for the H_{A} seeds in solution with an increase in the DP value of the P2VP was detected by DLS analysis (Figure S15). Thus, with an increasing DP value for the P2VP block, even though the lengths of seeds appeared to be very similar (ca. 50 nm, Figure S3), their increasing hydrodynamic diameters in solution would be expected to lead to a decreasing number of H_{A} seeds adsorbed. An AFM height image of the composite “shish-kebab” supermicelles from N_{280}H_{120}-N_{280} triblock comicelles and H_{A,448} seeds was obtained (Figure 5(c)), and it can be clearly observed that the H_{A,448} seeds were attached only on the central H_{D} segments. A cross-sectional analysis (Figure S16) showed that the central H_{D} segments with the adsorbed H_{A} seeds were of similar height to that of the individual N segments. It appears in the AFM image that the H_{D} seeds were only distributed on the two edges of the H_{D} segment (Figure 5(c)). This could be explained by the fact that the H_{A} seeds and H_{D} segments were attached by the interaction between their corona chains, which permits appreciable freedom for the seeds to move around when the supermicelles are dried on a substrate.

In the next set of experiments, triblock comicelles N_{280}H_{120}-N_{280} with different lengths for the H_{D} segments (610 nm, 880 nm, and 3.2 μm) were mixed with H_{A,448} seeds in i-PrOH at hydroxyl / pyridyl mole ratio = 2 / 1. The lengths of the H_{D} segments were uniform and precisely controlled via living CDSA (Figure S3). Composite “shish-kebab” structures were observed by TEM (Figure 6(a, b) and Figure S14(a-c)), and the H_{D} segments were fully covered by H_{A} seeds.

![Figure 7](image_url)

Figure 7. TEM images (a-c), AFM height image (d) and schematic illustrations (e) of fence-like “shish-kebab” superstructures formed by adding PFS_{280-b-PtBA_{280}} unimers to the previous “shish-kebab” structure solutions in Figure 6. The lengths of H_{D} segments are (a) 600 nm, (b) 880 nm, and (c) 3.2 μm, respectively. Scale bars are 500 nm in (a-c) and 200 nm in (d).

The bound H_{A} seeds were found to be still active to living CDSA and addition of the PFS-b-PtBA unimers led to the formation of new N segments (Figure 7(e)). Interestingly, the subsequent growth of the N segments led to their mutual repulsion and resulted in a perpendicular alignment of the N-H_{D}-N comicelles relative to the cross supermicelle arms. This afforded hierarchical fence-like “shish-kebab” supermicelles with high uniformity in size, architecture and yield (Figure 7(a-c) and Figure S14(d-f)). AFM height image (Figure 7(d), Figure S17) was also obtained from the same sample. A cross-sectional analysis (Figure S17(c)) showed that the height of the central section of the supermicelles was around 30 nm, three times that of the individual N segments. This suggests that the N segments, which were grown from the H_{A} seeds, were distributed on both upper and lower sides of the N-H_{D}-N triblock comicelles.

Direct characterization of the supermicelles in solution was achieved using LSCFM analysis of fence-like “shish-kebab"
supermicelles with N segments labelled with red fluorescent BODIPY dyes. Red dye-labelled N segments were grown from the H₄ seeds by living CDSA. The LSCM image of the fluorescent fence-like “shish-kebab” supermicelles in i-PrOH solution is shown in Figure S18(a). The N-segments grown on the fence-like “shish-kebab” supermicelles appear to be randomly oriented in the solution state, as shown in Figure S18(b), instead of being coplanar on the TEM grids. This can be best appreciated from the fuzzy edge of the supermicelles in the LSCFM images.

Taking advantage of living CDSA, we were able to successfully prepare heptablock comicelles N₂₈₀₋₁H₁₂₀₋₁D₁₂₀ (500 nm)₁N₂₈₀₋₁H₁₂₀₋₁D₁₂₀ (3.2 μm)₁N₂₈₀₋₁H₁₂₀₋₁D₁₂₀ (500 nm)₁N₂₈₀. The length of each of the segments was precisely controlled. When H₄ seeds in i-PrOH were added, they were adsorbed onto the H₅ segments and formed heptablock fence-like “shish-kebab” supermicelles (Figure 8(a) and (c)). The addition of PFS-b-PtBA unimers led to the formation of heptablock fence-like “shish-kebab” supermicelles after overnight aging at 23 °C, as shown in Figure 8(b) and (d). Interestingly, when the heptablock fence-like “shish-kebab” supermicelles were characterized 20 min after the addition of PFS-b-PtBA unimers, not all the unimers had been consumed and grown into N segments from the H₄ seeds. As shown in Figure S19(a) and (b), the new N₁H₁₂₀-N₁H₁₂₀ triblock comicelles with short N segments were closely attached to the H₂ segments of the heptablock, clearly demonstrating the growth of the N segments.

Figure 8. (a, b) TEM images and (c, d) schematic illustrations of (a, c) heptablock composite “shish-kebab” supermicelles prepared by mixing H₄,₄₄₈ seeds (50 nm) with N₂₈₀₋₁H₁₂₀₋₁D₁₂₀ (500 nm)₁N₂₈₀₋₁H₁₂₀₋₁D₁₂₀ (3.2 μm)₁N₂₈₀₋₁H₁₂₀₋₁D₁₂₀ (500 nm)₁N₂₈₀ heptablock comicelles, and the (b, d) block fence-like “shish-kebab” supermicelles prepared by adding PFS₂₀₋₁-b-PtBA₂₃₀ unimers to the previous “shish-kebab” structure. Scale bars are 1 μm.

Lastly, from TEM images, we measured the length of the H₅ segments in the N-H₅-N triblock comicelles and also counted the number of H₄,₄₄₈ seeds that could be adsorbed. Over 300 “shish-kebab” supermicelles were measured and the data were plotted in Figure 9(a). From the plots, we could calculate the average length occupied by each H₄,₄₄₈ seed to be 38 nm. However, when N₂₈₀₋₁H₁₂₀ (50 nm)-N₂₈₀ triblock comicelles were used, no seed attachment was detected and solely a mixture of the seeds and triblock comicelles were obtained (Figure S20). Only when the length of H₅ segments was increased to about 80 nm were the H₄,₄₄₈ seeds able to be adsorbed onto the N₂₈₀₋₁H₁₂₀₋₁ (80 nm)-N₂₈₀ triblock comicelles (Figure 9(b)). However, instead of forming a one-to-one complex, two H₄,₄₄₈ seeds adsorbed onto each N₂₈₀₋₁H₁₂₀₋₁ (80 nm)-N₂₈₀ triblock comicelle (as can be seen clearly from the inset image in Figure 9(b)). A schematic illustration of this process is included in Figure 9(d). After the addition of PFS₂₀₋₁-b-PtBA₂₃₀ unimers, new N segments were grown from the two H₄,₄₄₈ seeds, as shown in Figure 9(c).

Figure 9. (a) Plot of length of H₅ segments versus the number of H₄,₄₄₈ seeds that can be adsorbed onto the triblock comicelles (the dashed line is the linear fit of these data points). (b) TEM images of the supermicelles prepared by mixing H₄,₄₄₈ seeds (50 nm) with N₂₈₀₋₁H₁₂₀₋₁D₁₂₀ (80 nm)-N₂₈₀ triblock comicelles. The hydroxyl / pyridyl ratio = 2 / 1. Shown in image (c) are the supermicelles obtained after adding PFS₂₀₋₁-b-PtBA₂₃₀ unimers to the sample shown in image (b). The schematic illustrations of these supermicelle samples are included in (d). Scale bar is 1 μm for the image and 200 nm for the inset.

Discussion

1. The effect of mole ratios of hydroxyl / pyridyl groups.

It is noteworthy that in the three hierarchical assembly cases explored in this study (H₅, PMVSOH homopolymer with H₄ seeds, H₅, PMVSOH homopolymer with N-H₅-N triblock comicelles, N-H₅-N triblock comicelles with H₄ seeds), the optimum mole ratios of hydroxyl / pyridyl groups (i.e. the ratio at which the yield of supermicelles was maximised) are different. For the case of H₅, PMVSOH homopolymer and H₄ seeds, the largest amount of precipitate was formed when the ratio was 1 / 1, suggesting that the full binding of all the interacting sites was optimum for the formation of aggregates. On the other hand, in the case of H₅, PMVSOH homopolymer and N-H₅-N triblock comicelles, when H₄ segments were confined by the two N segments at both ends, this ratio shifted to 5 / 1. This is presumably due the existence of the N segments, which partially shielded 2VP groups and resulted in steric hindrance between the triblock comicelles. This steric hindrance prevented the triblock comicelles packing closely, so that an excess of hydroxyl groups, and thus H₅, PMVSOH homopolymer, was needed. This can be best appreciated by a comparison of the TEM images shown in Figure S5(c), where the seeds were very densely packed in the large precipitates to maximize the interaction with H₅, PMVSOH homopolymer, and those for “shish-kebab” supermicelles, where two N segments were
grown onto the H₃ seeds attached to the triblock comicles, and the intermicellar packing was clearly lower (this especially clear in the inset image in Figure 1(c)). Similarly, when H₃ segments were confined by N segments and H₃ seeds were used, an excess of H₃ seeds was required to prevent the intermicellar interaction to form large bundles of triblock comicles, as shown in Figure S13. This requires that the ratio is further shifted to 1 : 2 for the formation of composite “shish-kebab” supermicelles from N-H₂-N triblock comicles and H₃ seeds.

2. Formation of “shish-kebab” supermicelles.

When the H₃ homopolymer PMVSOH was added to N-H₄-N triblock comicles, the PMVSOH chains appeared to function as a “glue” to enable the triblock comicles to assemble via H-bonding interactions. Meanwhile, the N segments confined the interaction to only the central H₃ segments, leading to the formation of colloidally stable supermicelles.

As P2VP chains are soluble in i-PrOH and interact with H₃ homopolymer, the strength of the H-bonding interaction will increase with the value of DP₁P₂VP, and this effect will promote aggregation of the triblock comicles to form supermicelles. On the other hand, increasing the DP₁P₂VP block leads to an increased steric effect, hindering triblock comicle aggregation. By manipulating these factors, different supermicellar aggregates or supermicelles were obtained.

When DP₁P₂VP = 448 and DP₁P₂BA = 170, the steric hindrance was insufficient to confine the H-bonding interactions and three dimensional aggregates were formed (Figure 2(c), Scheme 2(a)). When DP₁P₂BA was increased to 280 or 460, the strength of steric hindrance and the H-bonding interaction was more confined and thus “shish-kebab” supermicelles were obtained (Figure 1(c) and 2(b), respectively, and Scheme 2(b)). However, when DP₁P₂BA was further increased to 600, the steric hindrance was sufficient to partially or even fully shield the central H₃ segments from interactions that would cause aggregation and the block comicles remained as discrete structures in solution (Figure 2(a)).

Increasing the strength of H-bonding interaction (by means of an increase in the value of DP₁P₂VP) had the same effect as decreasing the steric hindrance. As is clear from the TEM analysis, when the strength of the attractive interaction was increased (and DP₁P₂BA was fixed to 280), the aggregation of the micelles was enhanced, and the triblock comicles formed individual supermicelles (DP₁P₂VP = 250, Figure 2(d) and Scheme 2(c)). “shish-kebab” supermicelles (DP₁P₂VP = 448, Figure 2(e) and Scheme 2(b)), and three dimensional aggregates (DP₁P₂VP = 760, Figure 2(f) and Scheme 2(a)). The morphologies of these supermicelles or aggregates were very similar to those obtained by varying the DP₁P₂BA value, suggesting that the “shish-kebab” supermicelles result from a delicate balance between attractive interactions (from the H₃ segments and H₅ PMVSOH homopolymer) and repulsive interactions (from the N segments). Only in cases where both interactions were of comparable strength were triblock comicles able to form “shish-kebab” supermicelles.

The attractive and repulsive interactions can, in principle, be enhanced by increasing the lengths of the H₃ and N segments, respectively. However, as shown in Figure 4(a) and Figure S11, increasing the length of N segments was not found to significantly influence the morphology of the resultant supermicellar aggregates. This is probably due to the fact that the confinement of H-bonding interactions originates only from the PtBA chains in close proximity to the H₃ segments and therefore is only influenced by DP₁P₂BA and not the length of N segments. In contrast, longer H₃ segments provide more interacting sites to bind with H₅ homopolymer (Scheme 2(d)), and the enhanced attractive interactions that result lead to the formation of multilayered bundles (Figure 3).

Finally, “shish-kebab” supermicelles were formed in i-PrOH solution, simply by mixing the N-H₄-N triblock and PMVSOH homopolymer together. Although the supermicelle structures were characterized by TEM after they were dried on grids, they were formed in the solution state. The results from our DLS experiments revealed an increase in hydrodynamic diameter from 230 nm for the triblock comicle N₁H₄₋₁PrOH (50 nm)-N₁H₄ (with N segment length = 450 nm) to 615 nm for the “shish-kebab” supermicelles. This result clearly demonstrated that aggregation occurred in the solution state rather than simply on drying during solvent evaporation. This is consistent with the results of LSCFM experiments on the formation of a range of supermicelles utilizing non-covalent interactions.

3. Formation of composite “shish-kebab” supermicelles

When H₅ seeds were mixed with N-H₂-N triblock comicles in i-PrOH, the seeds were adsorbed onto the central H₃ segments, due to the strong H-bonding interaction between the P2VP and PMVSOH corona chains. The number of H₅ seeds that can be adsorbed onto the triblock comicles is determined by two factors: the length of H₃ segments and the size of the H₅ seeds.

As shown in Figure 5, with an increase in DP₁P₂VP for the H₅ seeds, the number of seeds that can be adsorbed onto the same N-H₂-N triblock comicles decreased. In the case of H₅ segments with a length of 600 nm, when DP₁P₂VP = 250, (Figure...
approximately 30 $H_{A,250}$ seeds can be adsorbed; when $DP_{P2VP} = 448$, (Figure 5(b)), the value was ca. 14 $H_{A,448}$ seeds; and when $DP_{P2VP} = 760$, (Figure 5(d)), ca. 5 $H_{A,760}$ seeds were adsorbed (in each case the number of adsorbed seeds was counted from TEM images and averaged from more than 40 supermicelles). Thus, the average length of the $H_0$ segment that can be occupied by each $H_A$ seed can be calculated to be 20 nm, 43 nm and 120 nm, respectively. However, the hydrodynamic diameters of these seeds were measured by DLS to be 58 nm, 72 nm and 88 nm for $H_{A,250}$, $H_{A,448}$, and $H_{A,760}$, respectively (Figure S15). The trend obtained for the solvated seeds therefore agrees with that calculated from dried samples. However, due to the existence of multiple interacting sites between $H_A$ seeds and $H_0$ segments, the H-bonding interactions are likely to be sufficiently strong to make the adsorption of $H_A$ seeds irreversible. This presumably leads to a non-uniform packing of the $H_A$ seeds on $H_0$ segments, which is particularly clear in Figure 5(d). The inefficient packing would also help explain the lack of a more quantitative relation between the length of the micelle occupied, and the hydrodynamic size of a seed.

As expected, the number of $H_A$ seeds that can be adsorbed is determined by the length of $H_0$ segments. We measured over 300 composite “shish-kebab” supermicelles and plotted the number of $H_A$ seeds versus the length of $H_0$ segments in Figure 9(a). From the plots, it is clear that the number of $H_A$ seeds is proportional to the length of $H_0$ segments. From the slope, the average length occupied by each $H_A,448$ seed can be calculated to be 38 nm. However, if $N$-$H_0$-$N$ triblock comicelles with a short $H_0$ segment were used, only when the $H_0$ segment was at least 80 nm long could the $H_A$ seeds be adsorbed effectively. Furthermore, instead of forming one-to-one complex, two seeds were adsorbed onto each of the triblock comicelles. This could be explained by the fact that the $H_0$ segments were surrounded by PtBA chains which can partially shield the H-bonding interaction, as shown in Scheme 3(a). It is likely that the $H_0$ segments need to reach a minimum length to adsorb $H_A$ seeds and overcome the ability of the PtBA chains to effectively prevent H-bonding interactions (Scheme 3(b)). However, once the minimum length has been achieved, even if the corona chains are short (DP of PMVSOH is only 120), the $H_0$ segments still exist in three dimensions, and the seeds can then be adsorbed from different directions.

**Scheme 3. Schematic illustration of the factors influencing the $H_0$-bonding interactions of $N$-$H_0$-$N$ triblock comicelles.** (a) for a short $H_0$ segment; (b) for a long $H_0$ segment.

Significantly, the $H_A$ seeds adsorbed onto the triblock comicelles remained active for further living CDSA and addition of the PFS-b-PtBA unimer led to the formation of N segments and thus fence-like “shish-kebab” supermicelles. The number of N segments can be grown is determined by the number of seeds adsorbed and thus, as discussed in the previous section, the length of $H_0$ segments. On the other hand, the region where seeds can be adsorbed is determined by the position of the $H_0$ segments. Therefore, by careful design of the $H_0$ segment-containing block comicelles, we could produce fence-like “shish-kebab” supermicelles (Figure 7) or even block fence-like “shish-kebab” supermicelles (Figure 8).

**Summary**

We report an in depth study of the hierarchical assembly of BCP micelles into complex higher-level structures via the use of H-bonding interactions. Monodisperse cylindrical block comicelles bearing H-bonding central segments (with either donor $H_0$ or acceptor $H_A$ coronal functionality) and non-interactive end segments (N) were prepared via living CDSA. The lengths and the locations of these segments were precisely controlled. The cylindrical block comicelles were the assembled in i-PrOH via H-bonding interactions and various interesting and complex supermicellar structures were obtained. We focused on the formation of “shish-kebab” supermicelles from N-$H_A$-$N$ triblock comicelles and the $H_0$-based homopolymer, or N-$H_0$-$N$ triblock comicelles and $H_A$ seeds, and, in the latter case, the derivative fence-like “shish-kebab” supermicelles. Their formation can be rationalized by the influence of two factors: the attractive H-bonding interactions between the P2VP and PMVSOH chains, and unfavorable steric repulsions from the non-interacting PtBA chains.

Although the methodology described here was based on PFS-containing BCPs, it should be applicable to the emerging group of other crystalline-coil BCPs and related molecular species that undergo seeded growth processes analogous to living CDSA. This study demonstrates avenues with which to construct new micron-scale supermicellar structures via hydrogen bonding. In principle, functional structures may be generated by taking advantage if the tailorable spatial organization of different coronal chemistries. This may provide a powerful method for the preparation of complex micron-scale structures with a variety of applications.

**ASSOCIATED CONTENT**

**Supporting Information.** Experimental details and additional results. This material is available free of charge via the Internet at http://pubs.acs.org.

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