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Hyperbranched Polymer Vesicles: from Self-Assembly, Characterizations, Mechanism, and Properties to Applications

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Hyperbranched Polymer Vesicles: from Self-Assembly, Characterizations, Mechanism, and Properties to Applications†

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Vesicles, including lipid vesicles, surfactant vesicles, as well as polymer vesicles, have been extensively investigated over the past fifty years. Among them, polymer vesicles have attracted more and more attentions for their low permeability, superior stability and toughness, in addition to the numerous possibilities for tailoring physical, chemical and biological properties. Polymer vesicles are generally fabricated through the self-assembly of amphiphilic polymers with a linear architecture. Recently, as a representative polymer with a highly branched three-dimensional architecture, hyperbranched polymers have also exhibited great potentials in preparing vesicles. The resulted hyperbranched polymer vesicles, defined as branched-polymersomes (BPs), have shown unique properties, such as giant and easily-tuned vesicle size, facile functionalization, special formation mechanism, appealing solution behaviours, *etc.*. In this *tutorial review,* ten years' advances in BPs have been summarized since their first discovery in year 2004, including the syntheses of vesicle-forming hyperbranched polymers, self-assembly methods, self-assembly mechanisms, as well as the special properties. In addition, the cytomimetic, biomedical and other initiatory applications of BPs are also included.

1. Introduction

Vesicles, possessing an interior aqueous volume separated from the exterior solution by a membrane composed of amphiphilic molecules, have been vastly investigated in living and artificial systems. The three key factors, namely the membrane, interior aqueous volume, and exterior solution, are all very important for vesicles. Firstly, the vesicle membrane is formed by the self-assembly of amphiphilic molecules, that is, molecules with a dual hydrophilic-hydrophobic character. Generally, according to the number of lamellae in each vesicle, vesicles can be divided into unilamellar vesicles and multilamellar vesicles. Furthermore, unilamellar vesicles can be commonly classified into monolayer and bilayer vesicles based on the number of molecule layers arranged in the lamella. In monolayer vesicles, the vesicle lamella is composed of a single molecule layer with segregated hydrophobic part and hydrophilic part. In bilayer vesicles, the lamella is composed of two molecule layers, and the hydrophobic parts of both layers arrange in the centre of membrane to form the hydrophobic phase, while the hydrophilic parts expose to aqueous medium to reduce surface energy. Secondly, the encapsulated volume or lumen provides a relatively steady environment, which means a lot for lives' vital reaction and artificial applications. Vesicles with different interior volume have been well studied, such as small unilamellar vesicles (SUVs, <100 nm), large unilamellar vesicles (LUVs, 100-1000 nm) and giant unilamellar vesicles (GUVs, >1 μm). Thirdly, the exterior solution is also important because it serves as the medium for material, energy and information exchange inside vesicles.

To date, numerous amphiphilic molecules have been reported to self-assemble into vesicles in solution. According the chemical structure of incorporated molecules, vesicles can be classified into lipid vesicles (liposomes), surfactant vesicles and polymer vesicles (polymersomes). As the first attempt at artificial vesicles, liposomes made from lipids with a small molecular weight (principally less than 1000 Da) have been extensively investigated since the first liposome reported in the 1960s.[1] On one hand, liposomes have the advantages such as similar structure with living cells' membrane, innocuousness of liposome phospholipidic components, *etc.*, thus have been widely used as instructive models of self-assembling colloids, study models for the origin of life, as well as vehicles for pharmaceutical, diagnostic, and cosmetic agents.[2,3] On the other hand, they also have disadvantages of relatively poor physical and chemical stability, and toxic residual traces left by the preparation procedure, which limit their applications.[4]

As an alternative kind of vesicles prepared from small molecules, surfactant vesicles are believed to be the simplest functional membrane models.[5] Surfactant vesicles, also known as "synthetic vesicles", are constructed from synthetic surfactants. The first surfactant vesicles were found by Gebicki and Hicks through shaking thin films of oleic and linoleic acid in aqueous buffer to yield closed bilayers, and thus termed as "ufasomes".[6] From then on, lots of efforts have been made to investigate the properties and applications of surfactant vesicles. In general, compared to liposomes, surfactant vesicles are not sensitive to hydrolysis and oxidation, that is, they are more chemically stable than liposomes. Moreover, surfactants are much cheaper and

easier to store than phospholipids, and they are facile to be modified due to the chemical variety of surfactants. However, the biggest disadvantage of surfactant vesicles may lie in the permeability to low-molecular-weight molecules, which hinders their further biomedical application as vehicles.[4]

Polymer vesicles, which are absolutely essential members of vesicle families, have attracted more and more interests of researchers in chemistry, physics, life science, and material science during the past twenty years. The first example of block copolymer vesicles was observed by Eisenberg and co-workers. They obtained vesicular aggregates through the self-assembly of linear polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA) diblock copolymers with a very short hydrophilic segment and thus termed as "crew-cut" micelles.[7] Later, Disher *et al.* observed another kind of polymer vesicles formed by linear polyethyleneoxide-*b*-polyethylethylene (PEO-*b*-PEE) diblock copolymers with a similar hydrophilic fraction to liposomes and thus coined as "polymersomes".[8] Compared with lipid and surfactant vesicles, polymer vesicles show a superior stability and toughness, lower permeability, and meanwhile, offer numerous possibilities for tailoring physical, chemical and biological properties. On the other hand, however, polymersomes have their weaknesses, for example, the low membrane fluidity.

Dendritic polymers, including dendrimers and hyperbranched polymers (HBPs), are the fourth generation of polymer architectures following the linear, branched, and crosslinking polymers. They have a three-dimensional (3D) highly branched globular structure, and special properties of large population of functional groups, lower solution or melt viscosity, smaller hydrodynamic radius, no or lower chain entanglement and better solubility when compared with linear polymers.[9,10] Vesicles prepared from amphiphilic dendritic polymers were firstly observed by Meijer and co-workers in the self-assembly of a series of "linear-dendrimers".[11] After that, many kinds of dendrimer-containing polymers, such as multi-arm dendrimers and "Janus" dendrimers, have been utilized to construct vesicles. These dendrimer structures, as well as the unique properties of so-formed vesicles, have been summarized by Percec *et al*.[12] Vesicles prepared from HBPs were firstly reported by Zhou and Yan by using a series of amphiphilic hyperbranched multi-arm copolymers in year 2004, and the obtained vesicles were termed as "branched-polymersomes" (BPs).[13] Since then, BPs constructed from hyperbranched multi-arm copolymers, "linear-hyperbranched" block copolymers, "Janus hyperbranched" block copolymers as well as other HBPs, have been well investigated.

Up to now, BPs have displayed special properties including good stability, low permeability, tailoring properties due to many functional groups, and good fluidity owing to the less entanglement among polymer chains. Besides, some other property advantages, such as the simple preparation methods, easily tuned and micro-scaled size, facile-functionalization abilities, *etc.*, have also been disclosed. In a word, BPs have a combined property of traditional surfactant and block copolymer vesicles. However, to date, there has not a review specific on BPs been published. In order to help catch an insight to this new kind of polymer vesicles, this *tutorial review* will summarize the first 10 years' works (2004-2014) on BPs from the aspects of their syntheses, self-assembly, characterizations, formation mechanisms and properties. In addition, the cytomimetic, templating and biomedical applications of BPs are also included.

2. What are BPs

Hyperbranched polymers (HBPs) are composed of dendritic units, linear units and terminal units distributed randomly along the polymer backbones. The branches are stretched into all directions, resulting in a divergent three-dimensional (3D) highly branched globular structure with a large number of terminal functional groups at the periphery. When compared with dendrimers, HBPs possess another advantage of facile one-pot preparation in a massive scale. As a result, HBPs have attracted increasing interests both in academia and in industry. Up to now, HBPs with different composites, degrees of polymerization (DPs), degrees of branching (DBs), as well as topological architectures, have been extensively synthesized and well-reviewed.[9,10,14-17] However, the solution self-assembly of amphiphilic HBPs, in which HBP are served as hydrophobic or hydrophilic block, had attracted little attention before 2004. In year 2004, Zhou and Yan first report the self-assembly of amphiphilic hyperbranched multi-arm copolymers into macroscopic multiwalled tubes millimeters in diameter and centimeters in length.[18] Since then, amphiphilic HBPs have demonstrated unique characteristics or advantages in self-assembly including controllable supramolecular morphologies and structures, special properties, characteristic self-assembly mechanisms, and facile functionalization processes. Morphologies emerged in the self-assembly of amphiphilic HBPs include micelles, fibers, tubes, honeycomb films, as well as BPs.[19-21]

BPs, simplified from branched-polymersomes, were first reported in year 2004 by the self-assembly of an amphiphilic hyperbranched polyether.[13] Like liposomes and polymersomes, this kind of polymer vesicles can be directly formed in water with a bilayer or a monolayer structure and with a size from 5 μm to 100 μm depending on polymer compositions in spite that the vesicle-forming polymers are highly branched in nature and have a higher hydrophilic fractions over 60%. Thus, to discern these vesicles from polymersomes, branched-polymersomes were coined at that time to stress that the structure of these vesicles is similar to polymersomes but they are generated from branched polymers rather than from linear polymers.

3. How to prepare and characterize BPs

The first consideration for BPs concerns the vesicle-forming amphiphilic HBPs, the self-assembly methods and the characterizations. In this section, we will summarize details of BPs from the above three aspects according to the reported literatures.

3.1 HBPs incorporated in BPs

In general, amphiphilic HBPs which self-assembled into vesicles in solution can be classified into four categories according to the topology of the polymers, that is, "hyperbranched" homopolymers, "hyperbranched multi-arm" copolymers, "linear-hyperbranched" block copolymers and "Janus hyperbranched" block copolymers (Fig. 1).

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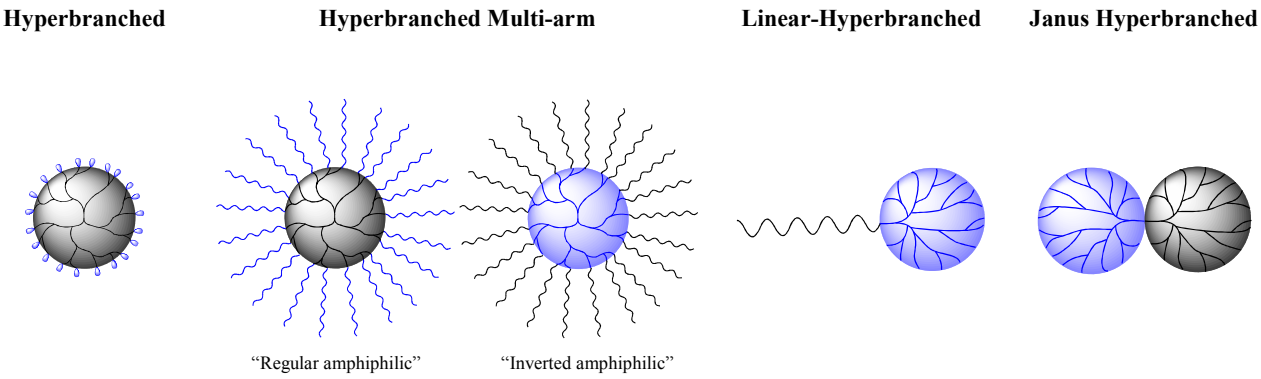

Fig. 1 Schematic representation of incorporated HBPs in BPs with different topologies. Black blocks are hydrophobic while blue ones are hydrophilic.

Hyperbranched homopolymers: As mentioned above, HBPs are 3D globular molecules with a large number of terminal functional groups at the periphery. These peripheral terminal groups can be readily modified by other groups with an opposite hydrophilicity to endow the whole polymer with amphiphilicity. This strategy will lead to amphiphilic HBPs with a hyperbranched core and modified end groups, that is, end-modified hyperbranched homopolymers (HBHs). For example, Zhou and co-workers modified the terminal hydroxyl groups of commercially available hydrophobic hyperbranched polyester of Boltorn Hx (x=20, 30, 40) (Fig. 2a) into polar carboxyl groups (Hx-COOH) and when self-assembled in water, pH-responsive vesicles were obtained in this way.[22]

"Hyperbranched multi-arm" copolymers: Similarly, the terminal functional groups of hyperbranched polymers can also be modified by other linear polymer chains or oligomers with an opposite hydrophilicity, thus resulting in amphiphilic polymers with a hyperbranched core and many linear arms, called as "hyperbranched multi-arm" copolymers (HMCs). To achieve this goal, either "graft to" or "graft from" method have been utilized. In fact, HMCs have occupied the majority of literatures on the self-assembly of BPs probably due to the facile synthetic procedure. In addition, according to the hydrophilicity of hyperbranched core and linear arms, HMCs can be divided into structures of "regular amphiphilic" HMCs and "inverted amphiphilic" HMCs (Fig. 1).

"Regular amphiphilic" HMCs refer to HMCs with a hydrophobic hyperbranched core and many hydrophilic linear arms. For example, the first reported and most investigated amphiphilic HBPs for preparing BPs, HBPO-*star*-PEOs (Fig. 2g), are composed of a hydrophobic hyperbranched poly(3-ethyl-3-oxetanemethanol) (HBPO) core (Fig. 2b) and many PEO arms.[13] Recently, Wu and co-workers synthesized HBPs with a hydrophobic hyperbranched polyethylene (HBPE) core (Fig. 2e) and poly((2-dimethylamino)ethyl methacrylate) (PDMAEMA) arms, and then the amphiphilic hyperbranched multi-arm copolymer HBPE-PDMAEMA further self-assembled into vesicles in aqueous solution.[23]

On the contrary, "inverted amphiphilic" HMCs refer to HMCs with a hydrophilic hyperbranched core and many hydrophobic linear arms. For example, Zhou and co-workers synthesized a series of HMCs (HPG-C16) with same hydrophilic hyperbranched polyglycerol (HPG) cores (Fig. 2c) and different amount of hydrophobic alkyl arms (C16) through the "graft to" method.[24] They found that HPG-C16s with different alkyl grafting ratios could self-assemble into vesicles in THF or THF/water mixed solvents. As another example, Chen and co-workers synthesized a biodegradable amphiphilic poly(ethylene glycol)-polyethylenimine-poly(ε-benzyloxycarbonyl-L-lysine) (PEG-PEI-PLys(Z)) in which hyperbranched PEI (Fig. 2d) served as the hydrophilic core while linear PLys(Z) served as hydrophobic arms.[25]

"Linear-hyperbranched" block copolymers: Except for the common HBHs and HMCs based on the hyperbranched core, amphiphilic HBPs can also be prepared from polymers consisting of a linear chain attached to a hyperbranched block, known as "linear-hyperbranched" block copolymers (LHBCs).[17] For example, a supramolecular LHBC was synthesized and further self-assembled into vesicles in aqueous solution.[26] The supramolecular LHBC (C_n-*b*-HPG) was prepared through the noncovalent coupling between adamantane-functionalized long alkyl chain (AD-C_n, n = 12, 18, 30) and hyperbranched polyglycerol grafted from β-cyclodextrin (CD-*g*-HPG) by the specific CD/AD host−guest interactions. The hydrophobic alkyl chains and hydrophilic HPGs endowed the LHBC amphiphilicity, and bilayer vesicles around 300 nm in diameter were obtained through a further self-assembly process.

"Janus hyperbranched" block copolymers: Very recently, HBPs with a "Janus hyperbranched" topology were synthesized to prepare BPs.[27] In this work, a hydrophobic HBPO with an apex of an azobenzene (AZO) group (AZO-*g*-HBPO) and a hydrophilic HPG with an apex of a β-cyclodextrin (CD) group (CD-*g*-HPG) were firstly prepared. Then an amphiphilic supramolecular Janus hyperbranched block copolymer (HBPO-*b*-HPG) was obtained through the host-guest interaction of CD and AZO groups. Further self-assembly of HBPO-*b*-HPG in water led to the formation of well-defined submicroscopic BPs.

In addition, expect for the aforementioned four topologies, crosslinked supramolecular polymers composed of CD/AD-modified hyperbranched PEI and AD/CD-modified fluorescent calcein dyes (Cal) also showed their potentials as building blocks for preparing BPs.[28]

In summary, amphiphilic HBPs have shown great potential in preparing vesicles. However, compared with the large number of

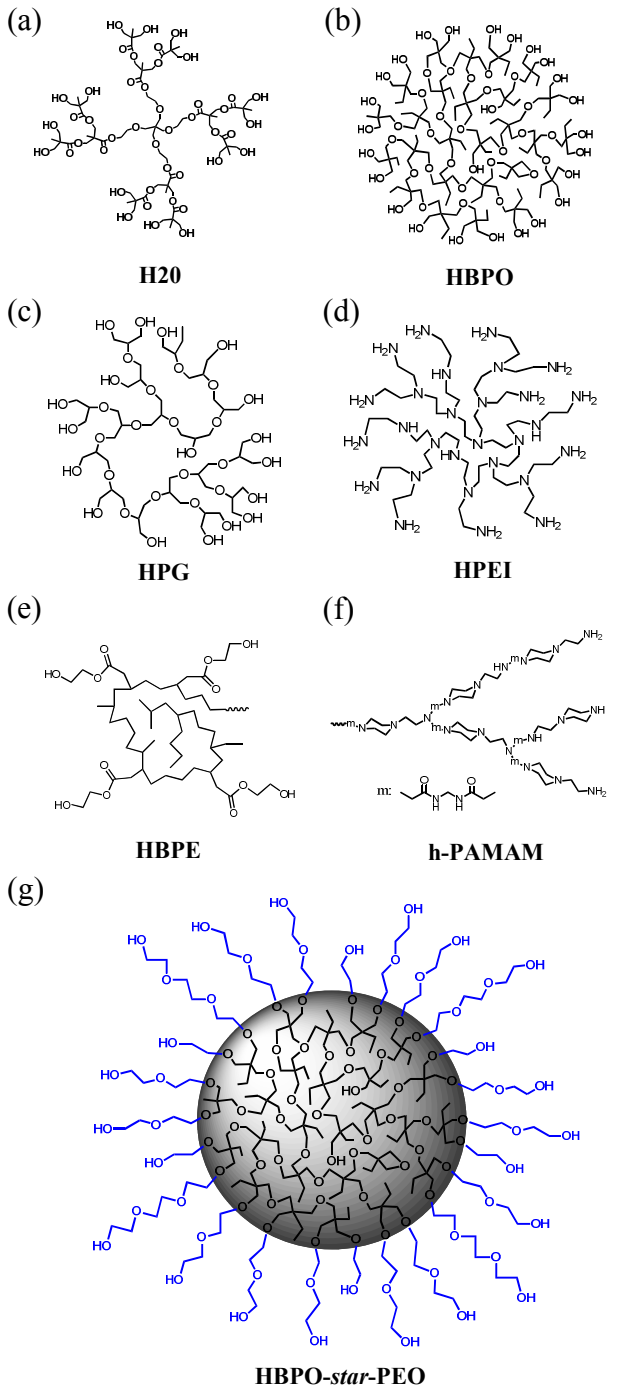

Fig. 2 (a)-(f)Chemical structures of the hyperbranched blocks incorporated in HBPs for fabricating BPs. (g) Fully outlined chemical structure of HBPO-*star*-PEO.

linear block copolymers which self-assembled into vesicles, the vesicle-forming hyperbranched polymers reported to date are greatly limited. Two main reasons probably count for this. The first one results from monomers for synthesizing amphiphilic HBPs. The synthesis of amphiphilic HBPs demands an AB_n monomer via single-monomer methodology (SMM) or two suitable monomers or a monomer pair via double-monomer methodology (DMM).[14] The limited kinds of such monomers lead to a much smaller amount of HBPs compared with linear block copolymers. Secondly, amphiphilic HBPs cannot be obtained through a "one-pot" synthetic process. Therefore, further modification of the hyperbranched precursors is necessary. Then, pathways for preparing amphiphilic HBPs should be carefully designed, leading to fewer suitable hyperbranched precursors.

3.2 Preparation methods

For vesicles prepared from lipids, surfactants and traditional linear block copolymers, many kinds of preparation methods have been reported, such as ethanol injection, directly dissolving, solid hydration (film hydration), membrane extrusion or ultrasonication, high pressure homogenization, template-directing, electroformation, microfluidic jetting and so on. Theoretically, these methods could also be used to prepare BPs due to the same amphiphilic nature. In fact, however, only minority of the methods have been applied to prepare BPs probably because the hydrophilicity, flexibility and intermolecular interactions of HBPs are different from other amphiphiles.

The first method is directly dissolving. In the self-assembly of HBPO-*star*-PEOs, Zhou and Yan adopted the simplest method by just placing the polymers into deionized water under stirring at room temperature.[13] Then, the amphiphilic polymers self-assembled into giant vesicles. A same method was adopted in Hx-COOH self-assembly and coassembly with poly-L-lysine (PLL), as well as the self-assembly of HBPE-PDMEMAs.[22,25,29] The self-assembly of supramolecular polymers constructed from AD-HPEI and CD-Cal or CD-HPEI and AD-Cal was also conducted by directly dissolving the corresponding polymers into water.[28]

Later, a "solid hydration" (or "film hydration") method was applied in the self-assembly of HBPs. The typical process was as follows: HBPs were firstly coated onto a vessel, and then water was added to induce the hydration of the polymer film to form vesicles. For example, in the self-assembly of HBPO-*star*-PEOs with very short PEO arms, giant large compound vesicles (LCVs) was obtained in this way.[30]

Subsequently, common solvent method for traditional linear block copolymer vesicles developed by Eisenberg was introduced into the self-assembly of BPs. The typical process was as follows: firstly dissolving polymers in a common solvent and then a selective solvent was added dropwise with agitation; finally the common solvent is removed by dialysis or evaporation to stabilize the aggregates. For BPs prepared from supramolecular "linear-hyperbranched" and "Janus hyperbranched" block copolymers mentioned above, a common solvent method was adopted in the preparation procedure.[26,27] For example, in preparation of BPs from supramolecular C_n-*b*-HPGs, both AD-C_n and CD-*g*-HPG was firstly dissolved in a common solvent (dimethylformamide, DMF), and water was added dropwise under stirring. When the solution exhibit opalescence, no more water was added and the solution was dialyzed against pure water to remove DMF. A stable aqueous solution of supramolecular C_n-*b*-HPG vesicles was obtained in this way. The coassembly of HBPO-*star*-PEOs with their derivatives (i.e., HBPO-*star*-PEO-CDs) or HBPO-*star*-PDMAEMAs were carried out by first mixing them together in common solvent, and then directly hydrated of the dried mixed polymers in water. [31,32]

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In addition, Chen and coworkers got giant BPs by directly pouring the THF solution of the amphiphilic HBPs (PEG-PEI-PLys(Z)s) into THF/H_2O mixture, and a following slow evaporation procedure of THF into air.[25] This preparation method is an inverted addition of polymer solutions into selective solvent, thus can be termed as "inverted common solvent" method for simplicity. It is noteworthy that other morphologies were obtained via different preparation method. For example, micelles appeared after a typical common solvent preparation method. However, if dropping the THF solution of the polymer into THF/H_2O mixture and THF was removed quickly by a rotary evaporator, rings came into being.

In summary, the reported preparation methods for BPs are limited to directly dissolving, solid hydration, common solvent and "inverted common solvent" method. Other methods, such as electroformation and microfluidic jetting, may also be applicable to prepare BPs with a narrow size distribution. Moreover, there are signs that relationships exist between the preparation method and the final self-assembled morphology of HBPs.

One more notable point is the self-assembly solvents. Analogous to traditional linear block copolymers, the same HBPs self-assembled in different solvents may result in different morphologies. Therefore, in order to obtain BPs, the effects of solvents should be taken into consideration. For example, Zhou and co-workers found that HPG-C16 with a relatively high alkyl grafting ratio formed unimolecular micelles in THF and giant vesicles in THF/water mixed solvents. However, the HPG-C16 with a low alkyl grafting ratio of 15.6% directly assembled into vesicles in THF and formed micelles in water.[24]

3.3 Characterizations

Characterizations are very important for BPs to provide evidences to the vesicular structure, the geometric parameters (i.e., size and membrane thickness), as well as the arrangement of molecules in vesicle membranes. Up to now, many kinds of characterization methods, such as laser light scattering (LLS), optical microscopy (OM), fluorescent microscopy (FM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), small angle X-ray scattering (SAXS) and nuclear magnetic resonance (NMR), have been used to evaluate BPs.

LLS: The LLS contains two kinds of techniques: static laser light scattering (SLS, Fig. 3a left) and dynamic light scattering (DLS, Fig. 3a right). From SLS measurements, the average radius of gyration (R_g) of the particles is allowed to be calculated according to the scattering intensity at different angles. From DLS results, the average hydrodynamic radius (R_h) can be obtained based on the experimental correlation functions. The ratio R_g/R_h can provide rough information about the morphology of the aggregates by comparing the experimental values with the theoretical ones. That is, the theoretical value of R_g/R_h for a uniform sphere is 0.774/1, and a polymer coil is 1.50/1, while a hollow sphere is 1.0/1. An experimental result of an approximate R_g/R_h ratio of 1.0/1 indicates the aggregates may be vesicles.[22,33,34] In addition, the DLS results can also provide other elementary information, for example, the size distribution, of vesicles besides the average hydrodynamic size.

OM: For micrometer-sized vesicles (giant vesicles), the observation by OM is direct and convenient. The hollow lumen and wall structure of vesicles can be directly identified in OM images (Fig. 3b, left). In addition, if hydrophilic dyes are encapsulated into the vesicles, the colored lumens can be easily discerned from the black wall by a color phase-contrast microscopy (Fig. 3b, right). Compared with electron microscope, the biggest advantage of optical microscopy may lie in the real-time observation. Therefore, OM has been extensively used in the observation of giant vesicles about their sizes, morphologies as well as time-dependent changes. For example, by using OM, Zhou and co-workers succeeded in realizing the real-time observations of membrane fusion, fission and a reversible vesicle-to-membrane transition of giant BPs.[35-37] In addition, the average size and size distribution of giant BPs can also be obtained through statistical analysis of a large number of particles from OM images instead of DLS measurements.

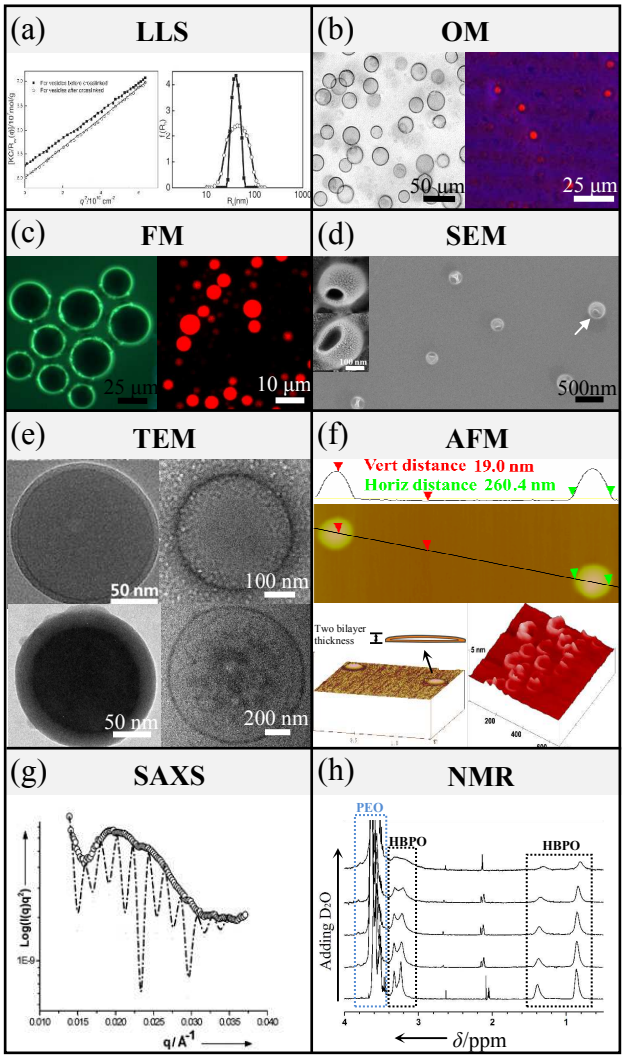

Fig. 3 The characterization of BPs by LLS (a), OM (b), FM (c), SEM (d), TEM (e), AFM (f), SAXS (g) and NMR (h). Reproduced from refs. 13, 26, 27, 31, 34 and 43 by permission of Wiley and the American Chemical Society.

FM: In order to better visualize the vesicular structure, BPs are often endowed with fluorescence either by chemically grafting fluorescent probes onto vesicle-forming HBPs or by physical

adsorption of hydrophobic probes into vesicle membranes.[22,29,31] As a result, the observed BPs often have a significant decrease in fluorescence intensity from the peripheral ring toward the centre of the sphere (Fig. 3c, left), indicating they possess a vesicular structure. Besides, if the vesicles encapsulate hydrophilic dyes, such as rhodamine B, into their lumens, the vesicles will exhibit as red-fluorescent particles (Fig. 3c, right). BPs labelled with different fluorescent probes can also be used to prove the fusion behaviour between them. For example, Zhou and co-workers labeled CD-functionalized BPs (CD-BPs) with rhodamine B and adamantine-functionalized BPs (AD-BPs) with dansyl. After mixing the red-fluorescent CD-BPs and green-fluorescent AD-BPs together for three days, bigger vesicles in orange were observed due to the colocalization of red and green, indicating the fusion between the two kinds of BPs.[38] This type of experiments, in which two kinds of BPs labeled with different probes, was coined as "double-labeling fluorescent assay". Sometimes, laser scanning confocal microscope (LSCM) was used instead of FM.[29]

SEM: SEM is an important tool in visualizing microscopic objects. However, it is not easy to use SEM to characterize vesicles. In general, only a solid particle morphology can be observed in the SEM image of vesicles. To better support the vesicular structure, holes should be generated or induced on the particles (Fig. 3d) by putting the samples under a high vacuum condition or under ultrasonication during the SEM measurements.

TEM: TEM is regarded as the most powerful tool in visualizing vesicular structures, especially for vesicles in nanoscale size. The TEM images of air-dried BPs generally show a contrast between the vesicle walls and their lumens (Fig. 3e, top left) to support the vesicular structure. For those BPs whose walls and lumens cannot be clearly distinguished, a staining method was often adopted. After staining procedure, if the staining agents have an interaction with the vesicle-forming HBPs, the agents are more likely to be incorporated into the polymer membrane. As a result, the vesicles are positively stained and the images will show darker walls than the lumens and surroundings because the higher density of mental atoms (of the staining agents) in membrane (Fig. 3e, top right).[27] If the agents have no interactions with the polymers, the wall will be lighter than the lumen and surroundings, thus exhibiting a negatively stained result (Fig. 3e, bottom left).[31] In order to exclude influences of drying procedure on BPs, cryogenic transmission electron microscopy (cryo-TEM) also is more powerful to prove the vesicular structure. Vesicle images obtained from cryo-TEM also show a contrast difference between the wall and inner pool (Fig. 3e, bottom right) although the resolution is often not as good as that of the conventional TEM images.

AFM: AFM is a very high-resolution type of scanning probe microscopy and describes the morphologies of the specimen surface. Therefore, as another visualizing method, AFM has been used to characterize vesicles generally after vesicle solutions are coated and dried onto a flat surface. As one of its advantages, AFM can measure the height as well as the length and width, thus can further display 3D profiles of the measured objects. According to literatures, the AFM results of BPs have the following characteristics. Firstly, from the 1D height profile of BPs, a high diameter-to-height ratio over 20 is often obtained (Fig. 3f, top). It is understandable because once vesicles are dried, the thin membrane is hard to support the vesicle framework and the vesicle will collapse onto the matrix. As a result, the diameter of vesicles measured by AFM is comparable to that of vesicles in solution, while the height will be rather small because it refers to the thickness of two collapsed vesicle membranes as shown in the cartoon of figure 3f (bottom left). Secondly, the AFM images of BPs generally show a flattened shape with round edges in a 2D view (Fig. 3f, middle) .[23,27] Thirdly, hollow lumens of BPs can be sometimes observed in a 3D view. As shown in Figure 3f (bottom right), the BP images have a concave crater structure for each particle, indicating the hollow sphere structure.[26] Moreover, holes can also be observed by AFM on the broken particles, which can directly prove the hollow lumen structure of vesicles.[26]

Therefore, from AFM results the following information can be concluded: Firstly, the vesicular structure can be confirmed. Secondly, the thickness of vesicle wall can be inferred. If combined with the molecular size of HBPs and OM or TEM results, detailed structural information, such as the number of lamellae of vesicles and the number of layers in each lamella, can be deduced. Furthermore, other supplementary mechanical properties of BPs, for example, ductility, can also be investigated by AFM.[26]

SAXS: SAXS is a technique where the X-ray scattering of the samples is recorded within a low angular range (0.1-10^o), which contains the information about the characteristic distances of partially ordered materials. Therefore, SAXS has been used to get the information on the number of lamellae as well as the thickness of the lamella in the vesicle walls.[39] In general, for vesicle suspensions, the SAXS result can be analysed by using the "flat sheet" approximation to spherical vesicles. In this model, the scattering intensity is proportional to the scattering vector and thickness. Thus the thickness of membrane can be calculated by fitting the SAXS result with curves based on the model.[31] For traditional liposomes and polymersomes, the SAXS signals are often strong (Fig. 3g). For BPs, however, the obtained SAXS results are not so good.[13,31] This is supposed to attribute to the irregularly globular structure of HBPs, which may lead to indistinct boundaries of molecular layers in the vesicle membrane.

NMR: NMR was often used to explore the molecular packing model inside BPs. The solution-state ^{1}H NMR signals are highly dependent on the mobility of groups in the solvent. As a result, the ^{1}H NMR spectrum can provide information about the group's mobility and the possible arrangement can be inferred from it.[13,23,26] For example, in the first work of BPs, Zhou and Yan attained the molecular packing information through ^{1}H NMR.[13] The HBPs were firstly dissolved in acetone-d_6 and D_2O was added gradually to induce the self-assembly process. A series of NMR spectra was recorded of solution with different D_2O content (Fig. 3h). The results showed that the signals for hydrophobic HBPOs were significantly decreased while those for hydrophilic PEOs kept strong along the addition of D_2O. It indicated the formation of a sandwich-like structure in vesicle membrane in which the PEO arms arranged on the outside and HBPO cores shielded in the interior.

Besides, some other measurements are also used to characterize the core-shell structure of BPs, for example, ζ-potential for surface-charged vesicles.[22,29,40]

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4. What's the mechanism for BP self-assembly

After the experimental characterizations, fundamental information for BPs, such as the size, membrane thickness, molecular packing model, and so on, has been disclosed. That is, we know exactly the chemical structure of the vesicle-forming HBPs, and the final BPs' geometric structure, and even the molecular packing model of HBPs in BPs. However, we cannot figure out the pathway and dynamics for the formation of BPs. This is mainly due to the experimental limitations that real-time observation of the self-assembly process is difficult to proceed at a molecular scale, and quenching the self-assembly process at every stage for observation is also hardly realized. As a result, the mechanism of BP formation remains as an important but unsettled problem.

Very recently, Zhou and co-workers have studied the mechanism for the formation of BPs by using computer simulations.[41] In this work, the authors adopted the dissipative particle dynamics (DPD) simulation due to its advantages of high computational speed, large integration time step, and covering a much longer time scale.

The simulation results for vesicles prepared from "regular amphiphilic" HMCs show that before self-assembly process begins, HMC molecules are random dissolved in water as unimolecular micelles. The subsequent self-assembly of HMCs into vesicles experienced four stages (Figs. 4a & 4b): Firstly, random distributed HMCs aggregated into small micelles; Secondly, the small micelles merged into membrane-like structures; Thirdly, membranes curved, bended and closed into small vesicles; Fourthly, the small vesicles fused into the final stable big vesicles. This mechanism is different with the process for traditional linear polymer vesicles and liposomes, in which "sphere micelle-to rod micelle" and "rod micelle-to-membrane" stages are additionally needed between the "sphere micelle-to-membrane" stage. Simulations for vesicles prepared from "inverted amphiphilic" HMCs were also carried out in this work, and a similar but faster process was found.

Furthermore, the packing model of HMCs in vesicle membrane was also investigated through DPD simulations. The results show that vesicles (so called normal vesicles) from "regular amphiphilic" HMCs should have two packing models. The HMCs with a short hydrophilic fraction prefer to form a bilayer structure, while those with a larger hydrophilic fraction prefer to form a monolayer structure. To clearly demonstrate it, the HMCs in the cross-section view of the vesicle are arbitrarily labeled with different colors. For the bilayer structure (Fig. 4c), it has been found two HMCs with a full "A-B" type microphase separation, in which each HMC spontaneously segregates into a hydrophilic arm part (A part) and a hydrophobic hyperbranched core part (B part), pack together in a head-to-tail way to form the bilayer. While, for the monolayer structure (Fig. 4d), only one HMC with an "A-B-A" type microphase separation is observed to span the cross section of the vesicles. This result agrees well with the experimental data of HBPO-*star*-PEO vesicles.[13,37] However, for vesicles from "inverted amphiphilic" HMCs (so-called reverse vesicles), only a bilayer structure is obtained, independent of the hydrophilic fraction. This is also in accordance with the experimental results.[29]

The abovementioned results indicate the DPD simulation is suitable to study the self-assembly of HBPs. In addition computer simulations serve as an alternative and powerful way to explore the details of vesicle self-assembly. Most importantly, some of these details, especially the self-assembly dynamics, are difficult to be obtained in an experimental level.

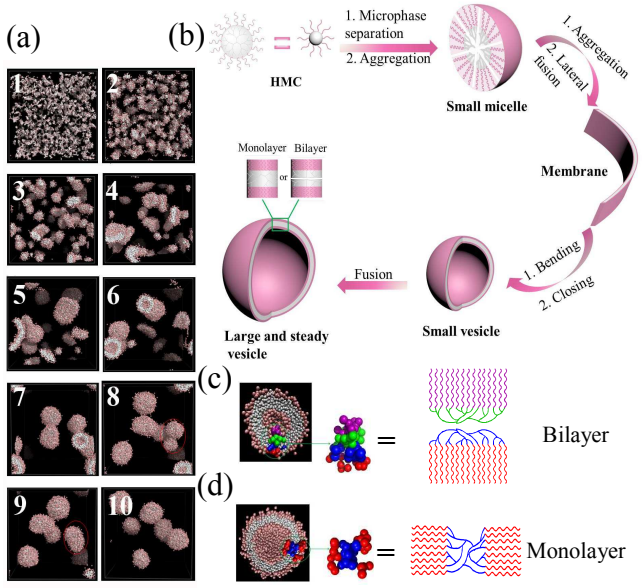

Fig. 4 Mechanism for the formation of BPs prepared from HMCs. (a) Time series of morphologies of normal vesicles prepared from "regular amphiphilic" HMCs with different simulation steps (from snapshot 1 to 10: 1.00×10^4, 1.00×10^5, 3.00×10^5, 5.00×10^5, 7.00×10^5, 1.00×10^6, 1.45×10^6, 1.50×10^6, 2.00×10^6 simulation steps, respectively). (b) Schematic representation of the evolution of the normal vesicles. (c) Cross-sectional views of normal vesicles with a smaller hydrophilic fraction. (d) Cross-sectional views of normal vesicles with a larger hydrophilic fraction. Reproduced with permission of Wiley from ref. 41.

5. What are the properties of BPs

As mentioned above, the investigations for BPs are only in an early stage. In spite of this, the investigations so far have already found many attractive properties, as well as some unique phenomena of BPs. This section will focus on the physical and chemical properties of BPs, such as geometric structure, stability, permeability, facile functionalization abilities, stimuli-responsive properties. Some unique phenomena found in BP systems are also highlighted.

5.1 Geometric structure

Once BPs are prepared, the first description for them should be their geometric structure, including size, size distribution, shape and membrane structure.

Size: Size is an appealing property for BPs, mainly for two reasons. The first one is BPs are often micrometer-sized, that is, giant vesicles. In general, the preparation of traditional giant polymer vesicles requires a specific technique, such as electroformation, pattern surfaces and microfluidic jetting.[8,42,43] While for BPs, giant vesicles are often obtained easily through a simple preparation method. For example, most of the reported HMCs, either with a "regular amphiphilic" or "inverted amphiphilic" structure, were found to self-assemble into giant BPs simply by direct dissolving or solid hydration methods. As

giant vesicles have great importance in studying the statistical physics and physical chemistry of amphiphilic membranes, as well as in investigating the properties and functions of biomembranes, the facile-prepared giant BPs may provide unique advantage in these studies.

The second reason is that the size of BPs can be easily tuned. For example, Zhou and co-workers synthesized a series of HBPO-*star*-PEOs with the same hydrophobic HBPO core and different hydrophilic PEO arms.[13,37] When the hydrophilic PEO molar fraction ($f_{hydrophilic}$) is changed from 66.6%, 80.0%, 85.7%, 91%, 93.8% to 95.2%, the average diamerter of BPs varies from 100 μm, 22 μm, 4 μm, 1 μm, 630 nm to 320 nm, respectively.. That is, the longer are the PEO arms, the smaller are the vesicles. In this way, BPs with different sizes ranging about three orders of magnitude from hundreds of nanometers to hundreds of micrometers can be easily obtained by altering the hydrophilic fraction of the HBPO-*star*-PEOs. It is quite rare to adjust the size of vesicles in such a broad range for only one polymer family.

There is one point should be noted that the hydrophilic fractions of HBPO-*star*-PEO copolymers are higher (>60%) than traditional "polymersomes". For traditional "polymersomes", a general rule is concluded that block copolymers with $f_{hydrophilic}$ ranging from 25% to 45% are supposed to form vesicles (for "crew-cut" micelles, $f_{hydrophilic}$<20%). However, block copolymers with $f_{hydrophilic}$ higher than 45% are supposed to form micelles.[44] Obviously, the HBPO-*star*-PEO vesicles do not obey the rules summarized from linear block copolymers, revealing a big difference between BPs and traditional polymersomes. In addition, the degree of branching (DB) of HBPs also have an influence on the formation of BPs.[45] Therefore, both the hydrophilic fraction and the DB of the HBPs should be taken into account for the fabrication of BPs with a desired size.

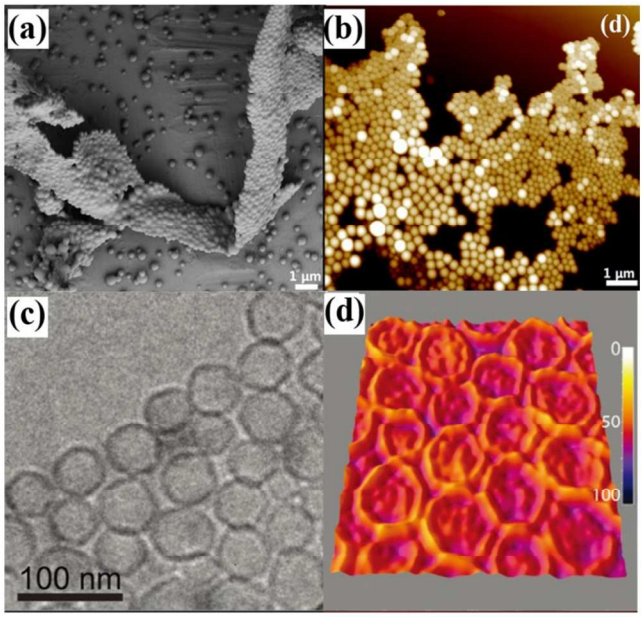

Fig. 5 Uniform vesicles fabricated from "Janus hyperbranched" polymers (a, b) and "Janus dendrimers" (c, d). Reproduced from ref. 27 and 46 by permission of the American Chemical Society and American Association for the Advancement of Science.

Size distribution: Another basic parameter to describe BPs is size distribution. For giant BPs, the size distribution is often broad due to the simple but crude preparation methods, while for smaller BPs, the size will be relatively narrow. It is reasonable because intervesicular fusion occurs more commonly for larger vesicles. An interesting result was observed that BPs with a nearly monodisperse size distribution were obtained in the self-assembly of "Janus hyperbranched" block copolymers via a common solvent method (Figs. 5a & 5b).[27] A similar result had already been reported in the self-assembly of "Janus dendrimers" by simple injection of the ethanol solution of the "Janus dendrimers" into water or buffer (Figs. 5c & 5d).[46] For liposomes and polymersomes, some specific techniques like templating method, membrane extrusion and microfluidic jetting have been used to get monodisperse submicroscopic vesicles. However, a simple cosolvent hydration method will generally lead to a broad size distribution for the obtained vesicles. The unusual phenomena on the vesicular self-assembly of "Janus hyperbranched" polymers and "Janus dendrimers" should be attributed to the special polymer architectures; however, the detailed mechanism is still not clear.

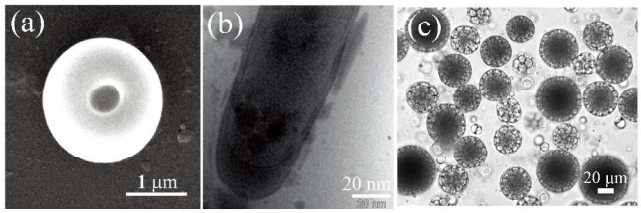

Fig. 6 Doughnut-like (a), tubular (b) and large compound (c) BPs. Reproduced from refs. 25, 28 and 30 by permission of the American Chemical Society and Wiley.

Shape: In addition to size and size distribution, geometric shape is also important for the description of BPs. Besides the most common spherical vesicles, doughnut-like and tubular BPs have also been discovered (Fig. 6a & 6b).[25,28] Moreover, a hierarchical self-assembly of BPs into large compound vesicles (LCVs) was also reported (Fig. 6c).[30]

Membrane structure: Hitherto, the reported BPs are limited to unilamellar vesicles. In these vesicles, most of amphiphilic HBPs arrange into a monolayer or a bilayer structure. A multilamellar structure was proposed for the mechanism of doughnut-like vesicles without further experimental evidences.[25] The different packing model of HBPs may attribute to the hydrophilic fraction or the topology structure of HBPs.[13,28,41]

5.2 Stability

The storage and chemical stabilities are excellent for nanoscopic or submicroscopic BPs, for example, the HBPO-*star*-PEO vesicles around 300 nm can be stored in the air without any changes in structure and morphology for at least half a year.[26,28,29,34] For giant BPs, the chemical stability is good determined by the polymers. The storage stability, however, is generally not as good as small BPs. This mainly contributes to the gravity effect of the giant vesicles to sink into the bottom of the container, and the further aggregation and fusion behaviours are also responsible for it. In addition, once the vesicle wall was cross-linked either covalently or non-covalent, the fusion behaviour is forbidden and the stability of giant BPs will be markedly improved.[28,29]

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5.3 Permeability

The permeability of BP membranes has not been investigated specifically, and the reported experiments show that some molecules can penetrate the vesicle membrane while others cannot. For example, rhodamine B is a middle-sized molecule with a molecular weight of 479 Da, and is found to be easily penetrated into the vesicle lumens through the membrane,[29,31] while a smaller molecule-ethylene diamine tetraacetic acid (EDTA)-cannot.[38] This difference obviously shows the penetration of molecules through BP membranes has no direct relationship with the molecular weight. Other factors, such as molecular structure and the polarity may count for this. Although no quantitative data have been shown, it is believed that the permeability of BPs is generally lower than the counterpart linear polymer vesicles. In addition, the permeability of BPs can be easily tailored by altering the chemical composites or molecular weight of the incorporated HBPs.

In addition, some other physical properties of BPs have been investigated or reflected. For example, the BPs prepared from supramolecular C_n-*b*-HPGs showed great ductility under external forces, indicating the vesicle membranes were flexible.[26] The high fluidity of BP membrane can also be reflected from the fusion and fission process of HBPO-*star*-PEO giant vesicles (details see below).[35,36]

5.4 Facile functionalization abilities

As mentioned above, HBPs are globular molecules with many functional groups at the periphery. This structural characteristic endows the so-formed BPs with facile functionalization abilities by utilizing those terminal groups. So far, the reported functionalization methods of BPs focus on two aspects: one is the preparation of biomacromolecules-immobilized BPs, and the other is the preparation of nanomaterial hybrid BPs.

Complex peptosomes: Peptide-decorated polymersomes, so-called peptosomes, have attracted more and more interests for their great potentials in drug delivery owing to the outstanding biocompatibility and biodegradability of polypeptides. In general, there are three pathways to prepare peptosomes: i) Self-assembly of amphiphilic polypeptide block copolymers. This method often needs complicate synthesises. ii) Template self-assembly by using a solid template. This method requires a further template-removal process. iii) Template self-assembly by utilizing a hollow template. This method generally bases on liposomes as the hollow templates, but the limited stability of liposomes will certainly restrict the mechanical properties of the so-formed peptosomes. Fortunately, by taking advantage of the numerous terminal functional groups of HBPs, BPs can be designed as an alternative candidate for preparing peptosomes.

Zhou and co-workers had found that BPs were obtained by the self-assembly of Hx-COOH (x=20, 30, 40) in which the terminal hydroxyl groups of the hyperbranched Hx were modified into carboxyl groups.[22] Then they further prepared a kind of peptosomes via one-step complex self-assembly of anionic Hx-COOH with cationic poly-L-lysine (PLL) through the electrostatic interactions between them, named as "complex peptosomes".[29] In this kind of peptosomes, the Hx-COOH formed BPs served as hollow templates and the polypeptide PLLs adsorbed to the vesicle surfaces. As a result, the "complex peptosomes" exhibited good biocompatibility endowed by the polypeptide, and meanwhile, excellent stability endowed by the BPs.

Nanocrystal hybrid vesicles: Recently, a new kind of functional vesicles in which metal or semiconductor nanocrystals are incorporated has attracted much attention for their unique optical, electronic and magnetic properties. Nanocrystal hybrid vesicles have been widely investigated for liposomes and polymersomes. As the large amount of terminal functional groups of HBPs might provide more possible sites for anchoring nanocrystals, hybrid BPs with a higher nanocrystal density are supposed to be obtained. For example, Zhang *et al.* prepared BPs by complex self-assembly of hyperbranched polyamidoamine (h-PAMAM, Fig. 2f) and linear polyacrylic acid (l-PAA).[39] After crosslinking the hydrophobic layer with glutaric dialdehyde (GDA) and removing the l-PAAs, the BPs were used to absorb metal cations and then in situ reduced them into nanoparticles by amino groups of h-PAMAMs. Interestingly, different metal cations, such as silver, gold and palladium, were found to be captured and further reduced by the BPs, indicating the potentials of BPs to prepare various hybrid vesicles in a facile way (Fig. 7a). In another case of hybrid BPs, Zhou and co-workers prepared thoil-modified BPs and then reduced Au cations into gold nanoparticles in the BP solution.[47] As a result, the reduced gold nanoparticles were chemically absorbed onto BP surface through the Au-S bondings. In both abovementioned cases, the hybrid BPs were giant vesicles and the hybrid density of metal nanocrystals on BPs was relatively high (Fig. 7b & 7c).

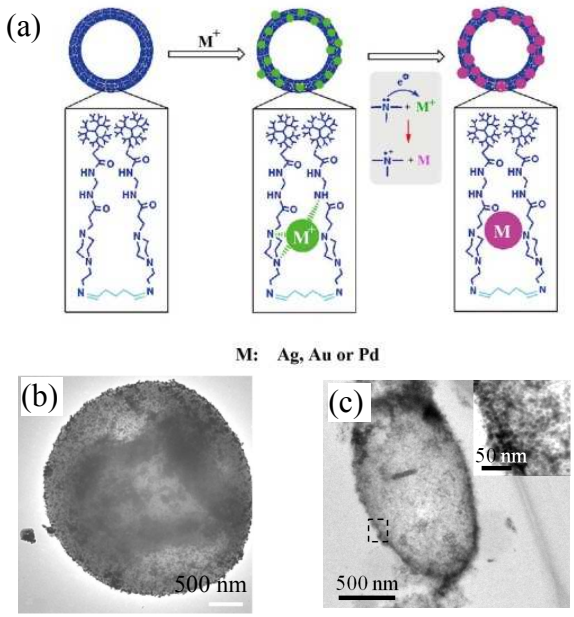

Fig. 7 Metal nanocrystal hybrid BPs. Schematic representation of the preparation of metal nanocrystal hybrid BPs (a). TEM images of palladium-hybrid BPs captured by Zhang *et al.* (b) and gold-hybrid BPs captured by Jin *et al.* (c). Inset in (c) shows a magnified image of the indicated area. Reproduced from refs. 39 and 47 by permission of the American Chemical Society and Royal Society of Chemistry.

5.5 Stimuli-responsive properties

For self-assemblies, an important research field is their

responsive abilities to the external stimuli, the so-called "smart" materials. For "smart" vesicles, they have attracted many interests because the changes in morphology, size, shape under external stimuli may provide a dramatic change in physical and chemistry properties, thus for applications such as "smart" drug or cargo release. The promising "smart" BPs are also prepared by introducing functional groups or segments into the vesicle-forming HBPs, the same way as the numerous similar works on traditional polymersomes. The following section will classify the "smart" BPs by the different stimuli factors, such as temperature, pH, additives and light.

Thermo-responsive BPs: Thermo-responsive vesicles have been widely investigated due to their great potential in biological and therapeutical applications. Generally, the principle for designing thermo-responsive vesicles is that one or more segments of the incorporated polymers will undergo a phase transition in solution by altering temperature, and the corresponding temperature is known as lower/upper critical solution temperatures (LCST/UCST). Therefore, thermo-responsive polymers, such as poly (*N*-isopropylacrylamide) (PNIPAM), are often served as at least one block in the vesicle-forming polymers.

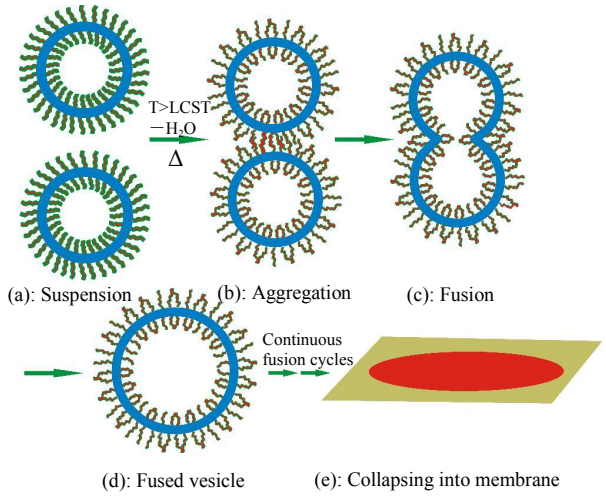

Fig. 8 A proposed model for BPs during the LCST transition. The red curves denote PEO arms, the blue rings denote the aggregated HBPO cores, and the green curves denote structured water around the PEO arms due to the ether-water hydrogen bonds. The green curves became thinner with the increase of solution temperature, which indicates the part destruction of structured water. Reproduced with permission from ref. 37. Copyright 2007 American Chemical Society.

PEOs also have LCSTs, and the larger are the PEO molecules, the lower are the LCSTs. In general, the LCST of PEOs is above 100 °C, even for large-molecular-weight PEOs. Therefore, it should be impossible for HBPO-*star*-PEO vesicles to exhibit a thermo-responsive behaviour in water. But in fact, they do. Investigations on the thermoresponsive phase transition behaviour of HBPO-star-PEO vesicles showed LCSTs ranging from 8 to 81 °C.[37] It is unexpected because the hydrophobic HBPO core has no thermosensitivity, and PEO homopolymers have a LCST above 100 °C. Further investigation showed that the vesicles underwent a reversible vesicle-to-membrane transition by changing temperatures alternatively above or below the LCST. Molecular mechanism had been proposed in this transition process, which was mainly contributed to the dehydration effect of PEO chains. That is, by raising temperature to a certain point, the dehydration of PEO chains could not maintain the repulsion between vesicles, thus leading to vesicle aggregations, and further fusion and morphological transition to membrane (Fig. 8). As a result, the vesicle solution turned into a turbid state and the corresponding temperature was the LCST of the vesicles. This is different from the traditional thermo-responsive polymersomes whose "smart" behaviours are generally endowed by the hydrophilic to hydrophobic (or in return) transition of polymer segments. In addition, the LCST of vesicle solution can be easily tuned with in a broad temperature span (over 70 °C) by changing the PEO length. This unique property and the thermo-responsive vesicle-to-membrane transition may allow the BPs for further applications in future, such as drug delivery and gene therapy.

Besides, BPs prepared from HBPE-PDMAEMAs are also reported to possess a thermo-responsive as well as a pH responsive behaviours due to the thermo/pH double responsiveness of PDMAEMA arms.[23]

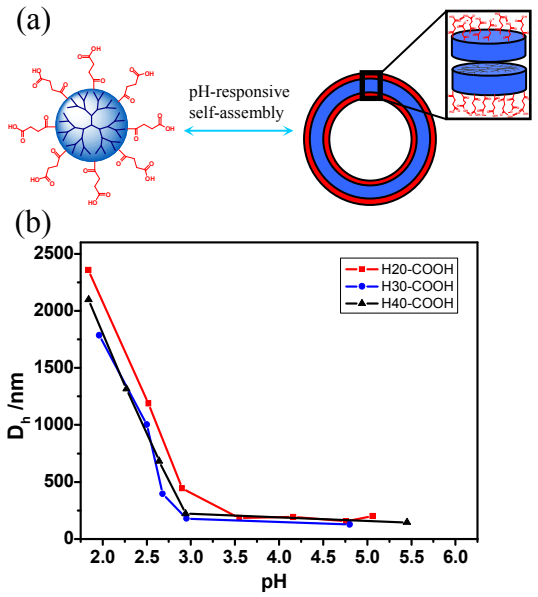

Fig. 9 pH-responsive BPs prepared from Hx-COOHs (x=20, 30 or 40). (a) Schematic representation of BP formation. (b) Hx-COOH vesicles D_h dependence on solution pH. Reproduced with permission of Wiley from ref. 22.

pH-responsive BPs: Vesicles in response to the external pH changes have received more and more attention in recent decades in order to develop their biological applications because of the numerous pH gradients in both normal and pathophysiological states of some biological systems. pH-responsive BPs was firstly reported by Zhou and co-workers by the self-assembly of Hx-COOH (x=20, 30 or 40) (Fig. 9a).[22] The hyperbranched Hx-COOHs were dispersed as unimolecular micelles in neutral and alkaline conditions. With a decrease of pH value of the solution, the self-assembly of Hx-COOHs underwent a morphological transition from unimolecular micelles into submicroscopic vesicles (3<pH<5.5) and then to giant vesicles (1.5<pH<3). In this way, the BPs showed a pH-responsive morphological transition as well as a remarkable size variation (from 200 nm to

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10 μm) (Fig 9b). It is noteworthy that such a broad size change of vesicles, controlled simply by altering the pH of the solution, is really unusual. Similarly, in the abovementioned case of BPs conducted from h-PAMAMs and l-PAAs, a morphological transition from solid micelles (pH<2.1 or >7.5) to vesicles (2.1<pH<7.3) can also be realized by adjusting the pH of the solution.

Another interesting pH-responsive behaviour was observed of BPs prepared from the co-assembly of HBPO-*star*-PEOs and HBPO-*star*-PDMAEMAs.[32] In a neutral or acidic condition (pH<10), isotropic binary vesicles were formed by the co-assembly of the two HBPs. With pH increasing (10<pH<12), the vesicles aggregated together to form LCVs due to the increasing hydrophobicity of vesicles introduced by the dehydration effect of PDMAEMA chains. Then an appealing microphase separation of HBPO-*star*-PDMAEMAs occurred in the vesicle membranes in solution with a higher pH (12<pH<14), leading to anisotropic vesicles with hydrophobic patches on them. Subsequently, the patches served as "binding sits" to trigger the intervesicular aggregation with different secondary self-assembly structure, such as linear vesicle chains, branched vesicle chains and cyclized vesicle chains, in a "polymerization-like" manner. After that, different types of tubes were formed by the fusion of membranes in those vesicle chains. In this way, the BPs underwent morphological transition, microphase separation, as well as "vesicle polymerization" behaviours in response to the external pH stimuli.

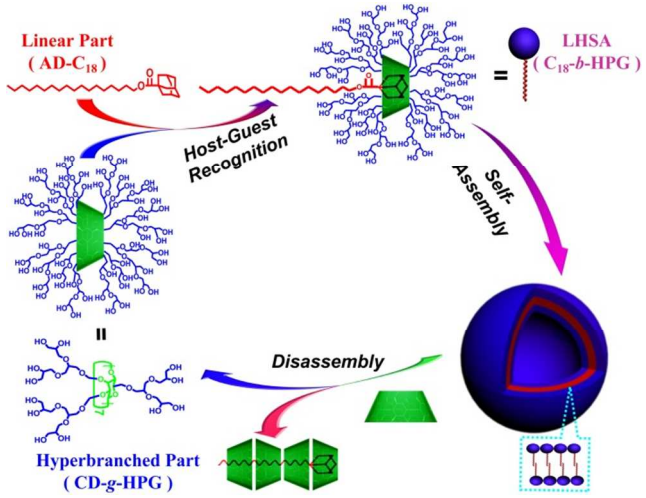

Fig. 10 Schematic representation of the self-assembly and "additive-induced" disassembly process of supramolecular "linear-hyperbranched" block copolymer C_n-*b*-HPGs. Reproduced with permission from ref. 26. Copyright 2012 American Chemical Society.

Additive-responsive BPs: Additives here refer to molecules which have an interaction with BPs, except for acids and alkalis. For example, an additive-responsive behaviour was observed in supramolecular C_n-*b*-HPG vesicles.[26] The amphiphilic supramolecular block copolymer C_n-*b*-HPGs self-assembled into BPs in water. When a competitive host molecule, *β*-CD, was added into the solution, the stronger complexation capacity between β-CD and AD-C_n led to disassociation between CD-*g*-HPG and AD-C_n. As a result, the amphiphilic supramolecular C_n-*b*-HPGs were destroyed and the so-formed BPs underwent a disassembly process into unimers (Fig. 10). Zhou *et al.* found that for HB2 vesicles, a fission behaviour was observed when glucose was added into the vesicle solution, which will be described in detail in the following section.[36]

Light-responsive BPs: Compared with other stimuli, light exhibits advantages in controlled morphological transition, aggregation and dispersion behaviour and release of encapsulated molecules, due to its weakest disturbance of the surroundings. The basic principle for designing light-responsive vesicles is incorporating photo-responsive chromophores into the vesicle-forming polymers. The chromophores will generate a conformational or structural change upon light irradiation, leading to a change of molecular size or hydrophilicity. This kind of light-responsive vesicle has been well investigated in linear polymers, but quite fewer works have been reported on BPs.

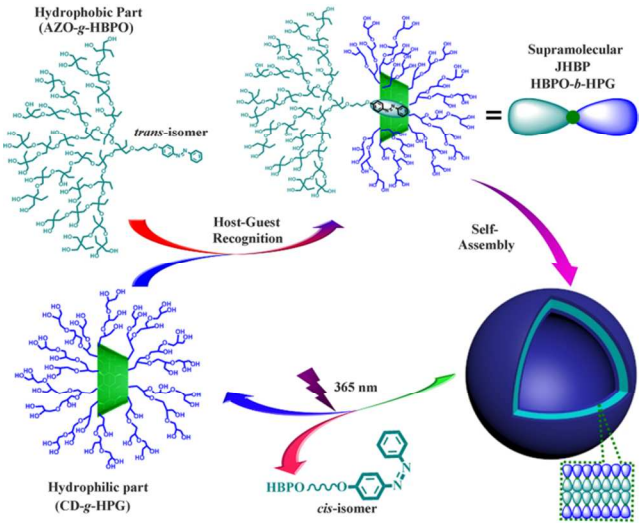

Fig. 11 Schematic representation of the self-assembly and "light-induced" disassembly process of supramolecular "Janus hyperbranched" block copolymer HBPO-*b*-HPGs. Reproduced with permission from ref. 27. Copyright 2013 American Chemical Society.

For example, Zhou and co-workers reported a new kind of BPs prepared from a supramolecular Janus hyperbranched polymer (HBPO-*b*-HPG).[27] The supramolecular HBPO-*b*-HPGs were constructed by AZO-*g*-HBPOs and CD-*g*-HPGs through the CD/AZO host-guest interaction under visible light, as mentioned above. As we know, when exposed to UV light, AZO groups will undergo a trans- to cis- isomerization. Trans-AZO groups can entry into the cavity of CDs to form host-guest complex due to the matched size and hydrophobicity, while cis-AZO groups cannot. As a result, the amphiphilic supramolecular HBPO-*b*-HPGs self-assembled into vesicles in water under visible light; once exposed to UV irradiation, the vesicles disassembled into unimers because the amphiphilic HBPO-*b*-HPGs was disassociated into hydrophobic AZO-*g*-HBPOs and hydrophilic CD-*g*-HPGs as a result of the destruction of CD/AZO host-guest interaction (Fig. 11). Another work on light-responsive BPs was reported by Zhou and co-workers to investigate a controlled vesicle-vesicle aggregation behaviour, which will be discussed in detail later.[31]

7. What are the applications of BPs

Although the applications of HBPs have cover many areas such

as drug delivery, macromolecular carriers, catalysis, sensors, surface engineering and biomimetic materials, the applications of HBP self-assemblies in solution have been far less investigated. Furthermore, the applications of HBP self-assemblies are mostly focused on micelles for biomedical applications.[21] For BPs, only a few works on their applications have been reported, mainly due to the short history of them. In this section, the cytomimetic, templating and biomedical applications of BPs have been summarized and commented.

7.1 Cytomimetic applications

The terminology "cytomimetic" chemistry, coined by Menger *et al.*, was used to describe the real-time shape transformations of vesicles in mimicking cellular morphological changes. In order to realize the real-time observation, micro-sized vesicles should be prepared as the first step. At the very beginning, liposomes are often selected in cytomimetic chemistry because their potentials in fabricating giant vesicles. In 2004, Zhou and Yan reported giant vesicles prepared from amphiphilic multi-arm HBPs in a simple way. The simple preparation process, good membrane fluidity and stability, especially the facile-tuned and micrometer-scaled size, make BPs ideal vesicles for "cytomimetic" applications. To date, the cytomimetic applications for BPs focus on two aspects: membrane fusion and fission, cell aggregation.

Membrane fusion and fission: As the first approach for cytomimetic applications by BPs, Zhou *et al.* realized the real-time observation of intervesicluar membrane fusion of BPs induced by ultrasonication.[35] The results showed that the whole fusion process experienced four successive steps: membrane contact; formation of centre wall; symmetric expanding of the fusion pore; complete fusion (Fig. 12). Further analysis suggested the fusion of BPs was corresponding with the proximity model for explaining lipid membrane fusion, in which perturbation generating defects were necessary. Subsequently, a vesicle fission behavior was monitored after adding glucose into the BP solution

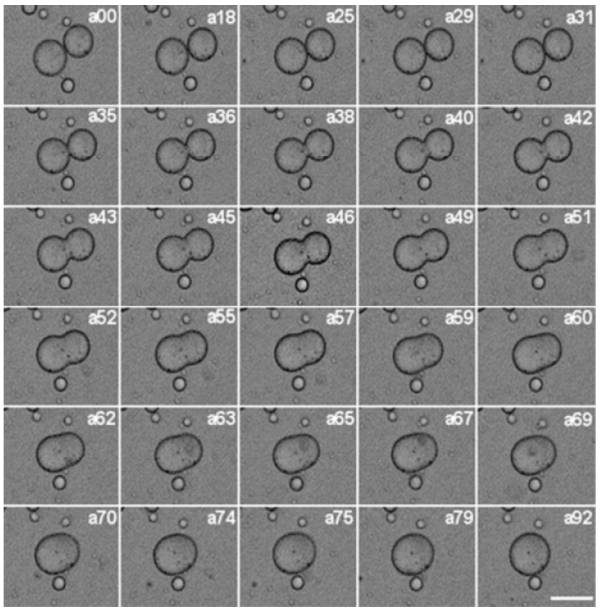

Fig.12 Time sequence of fusion images of two giant polymer vesicles. The number in the symbol labelled on each image denotes the elapsed time (in seconds), and the time of first image is set as zero. The scale bar represents 50 μm. Reproduced with permission from ref. 35. Copyright 2005, American Chemical Society.

by the same authors.[36] This glucose-induced vesicle fission process was caused by the osmotic pressure between the two sides of vesicle membrane, and realized by the cooperation of a mother vesicle and a daughter vesicle suspended inside. Thus the fission process can be regarded as a glucose-induced "cooperative fission". The fission and fusion processes are both absent of proteins, indicating proteins are not necessary in both processes. These works may help make it clear about the real mechanism of similar fission and fusion processes in lives.

Cell aggregation: Another important cytomimetic application of BPs is mimicking the cell aggregation behaviours. Cell-cell aggregation (CCA) is an important biological process which concerns a lot in hemostasis, inflammation, embryogenesis, immune responses and many other physiological processes. Although CCA is important, it is reasonably difficult to investigate this process because of the complexity of biomembranes. As an alternative and simplified model, vesicle-vesicle aggregation (VVA) is often selected to get an insight into CCA process due to the similar binary structure and properties between vesicle and cell membranes. BPs are ideal candidates in investigating VVA process for the similar micrometer-scaled size with cells and facile observation thereof.

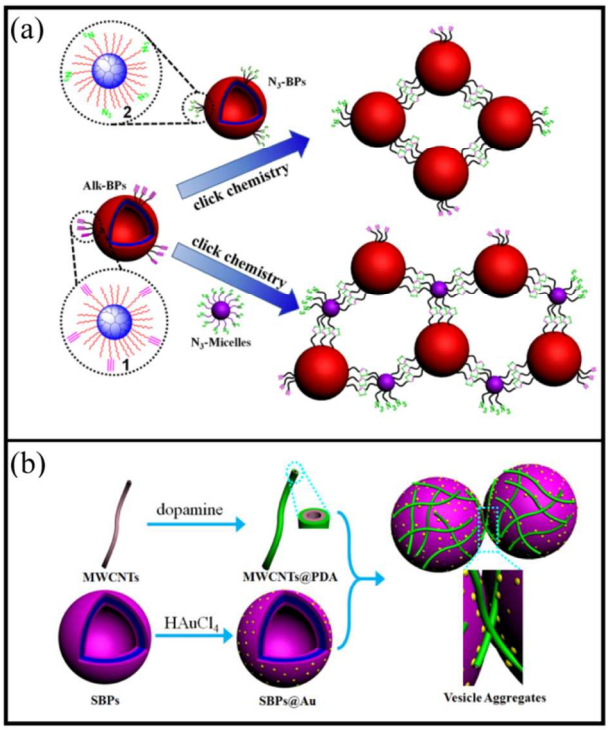

Fig. 13 Schematic representations of VVA process. (a) Aggregation of BPs triggered by click chemistry between alkynyl and azide groups. (b) Three-component vesicle aggregation triggered by adhesion interactions between AuNPs and PDA-coated MWCNTs. Reproduced from ref. 47 by permission of Wiley and the Royal Society of Chemistry.

For example, Zhou and co-workers prepared two kinds of vesicles, *β*-cyclodextrin (*β*-CD) functionalized BPs (CD-BPs) and azobenzene (AZO) functionalized ones (AZO-BPs), through the co-assembly of CD or AZO grafted HBPO-*star*-PEOs with unmodified HBPO-*star*-PEOs.[31] The so-prepared BPs are giant

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vesicles (5-10 μm) and can be further utilized to investigate the aggregation and dispersion behaviours between them. After mixing the two kinds of BPs together under visible light, the vesicles formed a coacervate phase consisted of a macroscopic three-dimensional (3D) network of densely packed vesicles due to the host-guest interaction between CD and AZO groups. When exposed to UV light, the disassociation between CD and AZO groups led to the re-dispersion of BPs. This transition can be repeated many times by alternatively exposing the solution to UV and visible light. In this way, they realized light-controlled aggregation and dispersion behaviours of BPs. Later, the same authors investigated VVA process through a stronger non-covalent interaction of CD/AD.[38] Then, a covalent-induced BP aggregation was also reported.[47] In this case, azide and alkynyl groups were loaded on the outer surface of two kinds of BPs, respectively (N_3-BPs and Alk-BPs) (Fig. 13a, upper). After mixing them together, the click reaction between azide and alkynyl groups happened. As a result, vesicle aggregation as well as vesicle fusion events and lateral phase separation on vesicle membrane occurred. In all the above mentioned three cases, vesicle fusion is observed after aggregation, especially in the latter two cases. This may be due to the host-guest interaction between the surface functional groups brought the vesicles too close to each other and further fusion event happened.

Then contrast experiments were carried on to check this deduction. Firstly, they found that if mixing Alk-BPs with N_3-micelles, vesicle aggregation happened while lacked further fusion events (Fig. 13a, lower).[47] The N_3-micelles acted as linkers which brought different BPs together, and meanwhile, acted as obstacles which restricted their further fusion. Secondly, a similar phenomenon was observed in a three-component (BPs, gold particles and nanotubes) "modular self-assembly".[47] In this case, VVA was realized by the interactions between gold-particle loaded BPs and polydopamine-coated carbon nanotubes (Fig. 13b). Interestingly, after mixing BPs with nanotubes, vesicle aggregated gradually and vesicle fusion, however, was also inhibited. It was supposed to attribute to the gaps consisting of nanotubes and gold particles between the adhered vesicles. Therefore, these results suggest the distance between two membranes plays an important role in vesicle fusion.

In summary, those above mentioned VVA process may help further understand the mechanism of CCA and provide an ideal model for investigating other processes or changes during CCA in a simplified manner.

7.2 Templating applications

BPs have also been selected as templates for fabricating nanoscaled objects and revealed some advantages. For example, in a very recent work, Zhou and co-workers developed a facile method for producing uniform polystyrene (PS) colloidal particles by using the bilayers of BPs as templates.[48] It was realized by firstly diffusing hydrophobic monomer of styrene, divinylbenzene (DVB) and photoinitiators of 2,2-dimethoxy-2-phenylacetophenone (DMPA) into the hydrophobic layer of giant HBPO-*star*-PEO vesicles, and then polymerized under UV irradiation. The nucleation and growth of PS particles were carried out in the wall of the BPs (Fig. 14). The uniform bilayer thickness and BP rupture process contributed to the monodisperse size distribution of PS particles. Moreover, the size of PS particles can be controlled from 60 to 150 nm by simply altering the feed ratio between the vesicle-forming polymer of HBPO-*star*-PEO and the monomer of styrene.

Vesicles is a hot research area in recent decades, however, most of the applications of vesicles in reported literatures are focused on encapsulation, drug delivery and mimic of cells. Differently, in this work, BPs, despite highly polydisperse size, have been used as templates to prepare small uniform PS particles. It represents a novel application of vesicles and should be extended to prepare other uniform nanoparticles in a large scale.

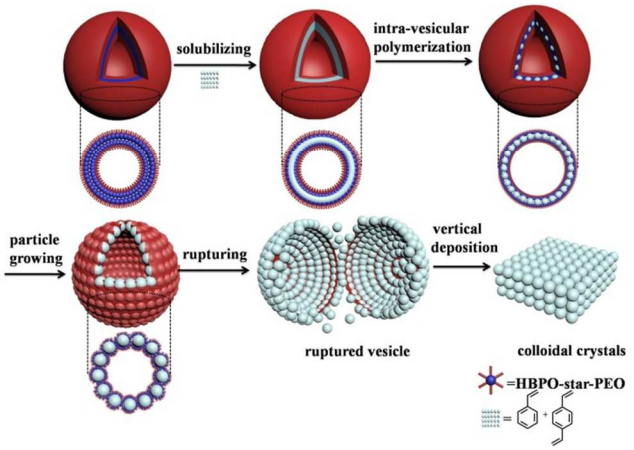

Fig. 14 Synthesis of monodisperse PS particles by vesicle bilayer templating. The hydrophobic HBPO layer of vesicles is in blue, the hydrophilic PEO coronae are in red, and the monomers and PS particles are in light blue. Reproduced by permission from ref. 48 by the Royal Society of Chemistry.

7.3 Biomedical applications

Recently, Frey and co-workers synthesized a series of cholesterol-anchored, HPG-based lipids, and found they can be easily integrated with other common lipids into vesicular structures.[49] This kind of liposomes has the following advantages: i) They are nontoxic due to the good biocompatibility of both HPGs and lipids. ii) The liposomes can be easily functionalized by modifying the functional groups of HPGs. As a result, such liposomes showed great potential for biomedical applications. For example, a kind of alkyne-modified HPG with cholesterol-anchored in one end was used as a functional component in the liposomes constructed mainly from cholesterol and egg phosphatidyl choline (EPC). Then the alkynes can be easily reacted with two kinds of N_3-fluorophores through the click reaction, resulting in dual labeling liposomes (Fig. 15). This dual labeling facilitated the study of cellular uptake and intracellular distribution of the liposomes. The result showed that HPGs and related compounds were readily fuse with cellular membranes, and can be used for the imaging of both cellular and endosomal membranes thereof.

HBPs are easy to functionalize liposomes or polymersomes through a co-assembly method to get sterically stabilized and biocompatible vesicles. In addition, some BPs themselves also demonstrate some potentials in biomedical applications. For example, HBPO-star-PEOs with different DBs in HBPO cores were synthesized by Zhou and co-workers. They found all polymers demonstrated good antifouling properties depending on the DBs.[50] Thus, it is believed the HBPO-star-PEO vesicles may

also have good antifouling properties. Nevertheless, the applications of BPs in biomedical areas are quite limited up to now.

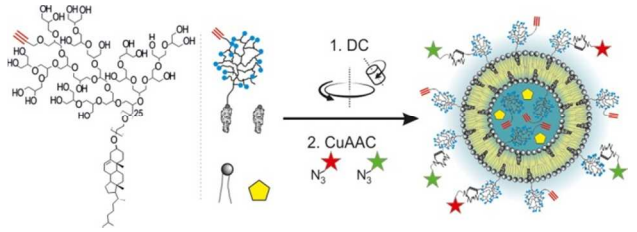

Fig. 15 Schematic representation of the preparation of hyperbranched polymer decorated liposomes. Reprinted with permission from ref. 49. Copyright 2014 American Chemical Society.

8. Outlook

In summary, this *tutorial review* has given an overview of the new emerging hyperbranched polymer vesicles (named as branched-polymersomes) from the aspects of synthesis, self-assembly, characterizations, properties and applications. Due to special structures and properties of hyperbranched polymers, branched-polymersomes have also demonstrated characteristic properties like special geometric structures, good stability and permeability, facile functionalization abilities, and smart stimuli-responsiveness. In addition, branched-polymersomes also show interesting applications in cytomimetic chemistry, templating synthesis and fabrication, as well as biomedical applications. These features make them become unique and important in spite that they are just a new member in the whole vesicle family. Nevertheless, there remain many unsettled problems of branched-polymersomes. Firstly, more hyperbranched polymers with different components or topological architectures are expected to be synthesized to extend the categories and functionalities of branched-polymersomes. Secondly, properties, such as stability, permeability and mechanical properties, of BPs should be investigated deeply and systematically in a quantitative way. Thirdly, the hybrid self-assembly of BPs with other components, such as proteins, DNA, quantum dots (QD) and other nanomaterials, are greatly limited, which should be explored in detail in order to get more functional vesicles. Fourthly, more advanced self-assembly methods such as "hierarchical self-assembly" and "modular self-assembly" with the building blocks of branched-polymersomes should be further developed to get more smart and complicated supramolecular structures, such as cell-mimetic or organ-mimetic self-assembled systems. Fifthly, more theoretical studies like computer simulations on the self-assembly and functionalization of branched-polymersomes should be performed to clearly disclose all the details inside. Finally, more applications, especially biomedical applications like drug delivery and gene therapy, should be further explored to promote the development of branched-polymersomes. We believe, branched-polymersomes will become more and more important in the future with all these issues being addressed.

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Key Learning Points:

1. The historical advances of vesicle family
2. The preparation and characterization methods of hyperbranched polymer vesicles
3. The special formation mechanism of hyperbranched polymer vesicles
4. The unique properties of hyperbranched polymer vesicles
5. The potential applications of hyperbranched polymer vesicles

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