Chapter 2
Nano-sized Polymer Structures via Self-assembly and Co-assembly Approaches

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Introduction

The amphiphilic copolymers in selective solvents self-assemble in a much similar fashion as the conventional low-molecular weight surfactants and naturally occurring lipids. They are composed of at least one solvophobic moiety and at least one solvophilic moiety (*hydrophilic* and *hydrophobic*, respectively, if the solvent is water). The difference in the solubility of the constituent moieties as well as the constraint imposed by the chemical linkage between them govern the geometry of the self-assembled structures, whereas the sequence of the latter is dictated by the proportions of the constituent moieties. At low concentrations, the self-assembled structures are discrete and well-separated, whereas upon increasing concentration, different liquid-crystalline phases are formed. The shape of the self-assembled structures varies from spherical to cylindrical to lamellar depending on the conditions; however, the main shape-determining factor is the ratio between the constituent moieties. For polymers, it is convenient to characterize the preferred aggregate morphology by the hydrophilic fraction, *f*. The relations between the preferred geometry of the self-assembled structures and *f* are presented in Fig. 2.1 [1].

The *critical aggregation concentration*, *CAC*, is a fundamental parameter defined as the concentration at which aggregates are formed. In other words, below the CAC only unimers, that is unassociated macromolecules, exist, whereas above the CAC multimolecular aggregates are in dynamic equilibrium with the unimers. The CACs of amphiphilic polymers are typically located in the low micromolar

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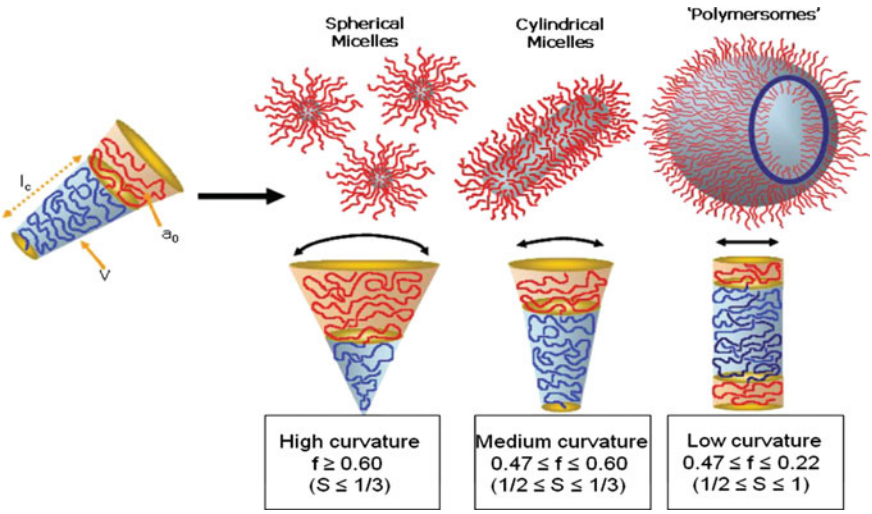

Fig. 2.1 Interrelations between the self-assembled structures formed by amphiphilic copolymers in aqueous solution and their hydrophilic fractions, *f*. The values of the corresponding surfactant packing parameter, *S*, are given in parentheses. Reproduced from [1] with permission from John Wiley and Sons

region and even lower, which implies greater stability and is advantageous for many applications since high dilution might be problematic for the conventional low-molecular-weight surfactants. The self-association can also be induced at a fixed concentration by manipulation of the solvent quality and environmental parameters. Thus, for copolymers that are composed of moieties exhibiting enhancement of hydrophobicity at certain temperature or pH, one can accordingly speak of critical temperature or pH of association.

The spherical micelles (Fig. 2.1) are the most extensively studied polymeric self-assembled structures. They represent the simplest and most widespread aggregate morphology. Cylindrical micelles as well as polymer vesicles (polymersomes) are less frequently observed (Fig. 2.1), whereas complex structures such as toroids, helices, multicore and multicompartment micelles, disks, tubules are considered as more or less exotic structures. Worth mentioning is the phenomenon of coexisting morphologies, which is believed to derive from a number of inherent for the synthetic copolymers characteristics, e.g., dispersity in molar mass.

Besides the critical aggregation concentration, temperature or pH and specific morphology, the polymeric self-assembled structures are characterized by particle molar mass and aggregation number, dimensional parameters such as hydrodynamic radius, radius of the core or membrane thickness for the polymersomes and disk-like micelles, radius of the cylindrical micelles and thickness of the corona, radius of gyration. A distinctive feature of the polymeric self-assembled particles is the *core-corona* structure. Both, the core and corona, can be considered as separate entities which are able to accommodate active substances of appropriate nature and to serve as

carriers. The core is composed of strongly entangled solvophobic chains, whereas the corona is built up of solvophilic chains, which can be nonionic or charged. Depending on the macromolecular characteristics, the corona can be thick and thus largely contributing to the overall particle dimensions or relatively thin. The core–corona interface is sharp if the core- and corona-building moieties are incompatible. Accordingly, for more compatible moieties a partial mixing of chains in the boundary region is expected. Last but not least, by carefully designing the copolymer composition, intelligent properties can be conferred to the whole aggregate.

Polymeric Nanostructures Prepared via Self-assembly and Co-assembly of Preformed Copolymers

General Remarks

Block copolymers tend to form nano-sized structures due to the differences in the physicochemical characteristics of each block. As a typical example, the case of an amphiphilic block copolymer can be discussed, where only one of the blocks is water soluble. In the aforementioned example, the hydrophobic blocks tend to aggregate in aqueous media, due to hydrophobic interactions, leading to the formation of a nano-sized aggregate where the hydrophilic blocks are extended to the solvent, stabilizing the nanostructure. There is a plethora of such self-assembled polymeric nanostructures in solutions that has been described in the literature, the ones observed in aqueous solutions being significantly more interesting for biomedical applications. Among them, there are some structures that can be characterized nowadays as common since they have been obtained in several systems. These include spherical core–shell micelles, worm-like or rod-like micelles, and vesicles or polymersomes. Some other nanostructures possess less common morphologies, namely toroidal, multicompartment or bicontinuous micelles. Some of these morphologies are shown in Fig. 2.2. In addition, the formed nanostructures can be (i) dynamic, i.e., exchange of block copolymer chain is possible, (ii) stimuli responsive, i.e., the nanostructures respond by changing their structural characteristics or fully disintegrate under external stimuli, such as temperature, pH, ionic strength, light, etc., (iii) kinetically frozen, i.e., exchange of chains is not possible by changing all experimentally relevant conditions in the normally accessible range of physicochemical parameters of the system, and (iv) chemically stabilized, i.e., they may contain covalently cross-linked cores or shells and their morphology/structure is "locked."

Since the literature on the common block copolymer micellar structures is vast, only a limited number of examples will be given, in order to outline the fundamental principles behind the self-assembly of macromolecular chains. Moreover, the so-called common polymeric nanostructures have been described in detail by a number of review articles and books, which are strongly recommended for further reading [1–4]. The story of self-assembly is tightly connected with the story of

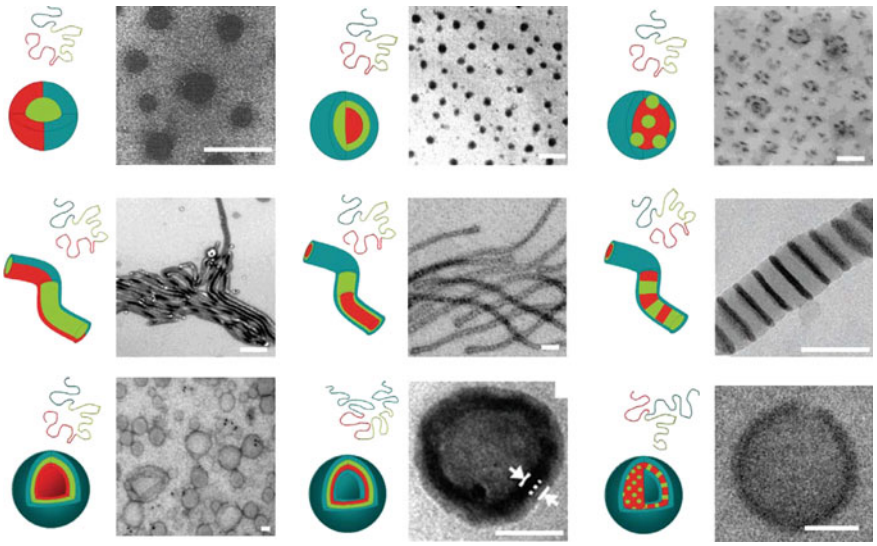

Fig. 2.2 Assemblies formed in selective solvent conditions by multiblock copolymers (from upper left to right): Janus spheres, core-shell spheres, raspberry-like spheres, Janus cylinders, core-shell cylinders, segmented cylinders, asymmetric (Janus) membrane vesicles, double-layer membrane vesicles, vesicles with hexagonally packed cylinders. Reproduced from [3] with permission from Elsevier

well-defined block copolymer synthesis, which started more than 50 years ago [5]. Meanwhile, a large number of research reports describe the effect of solvent quality in self-assembly, the connection between macromolecular characteristics and solution behavior and the effect of the environmental parameters, like temperature, salinity, and pH in the aggregation of block copolymers and the major role of polymer concentration to the self-assembly procedure. The effect of environmental parameters is one of the most appealing research subjects during the last years, because "responsiveness" is very important from a technological point of view, since it gives the opportunity to fine-tune the structures and their physicochemical properties by external stimulation of the system, a characteristic which is important for drug delivery and "switching" related applications.

Nanostructures Prepared from Stimuli-responsive Copolymers

The responsiveness of a block polymer is the key parameter for an application. Yang et al. [6] has described the potential application of a block copolymer as nano-sized drug carrier which offers fast release rate in the intestinal track. The block copolymer, namely poly(methyl methacrylate-co-methacrylic acid)-b-poly

(poly(ethylene glycol) methyl ether monomethacrylate) [P(MMA-co-MAA)-b-PPEGMA] tends to form spherical micelles upon dissolution in aqueous media with PPEGMA being the shell. Moreover, it has been found that its drug entrapment efficiency towards hydrophobic drugs reached to 90 %. The most important characteristic of the copolymer, however, is its selective responsiveness to the environmental pH. Thus, the copolymer was observed to keep its spherical micellar structure for pH lower than 5, but the micellar core can swell or even dissociate at pH values higher than 5. In an in vitro experiment, concerning the release of the drug ibuprofen, it was found that less than 20 % of the initial drug content was released in simulated gastric fluid (pH 1.2) over 12 h, but 90 % was released in simulated intestinal fluid (pH 7.4) within 6 h. The above behavior indicates an appealing candidate as drug nanocarrier, with selective release in the intestinal track. The above example clearly indicates that the responsiveness of a copolymer, and the better understanding of the relation between external stimuli and micellar response/structure is essential for the development of systems with appropriate efficacy.

Toward this end, the solution behavior of poly(2-(methacryloyloxy)ethyl phosphorylcholine)-b-poly(2-(diisopropylamino)ethyl methacrylate) (PMPC-b-PDPA) have been studied by Pearson et al. [7]. The dissociation constant (pK_a) for the conjugate acid form of the PDPA block was determined for a number of PMPC-b-PDPA copolymers with varying volume fractions of DPA over a wide range of temperatures. The polymer tends to form aggregates at pH values between 5 and 7.5. However, the size and the structure of the aggregates vary with the volume fraction of PDPA and temperature. The obtained data indicate a wide gamut of structures which can be formed from a single block copolymer, structures that are extended from simple spherical micelles to vesicles and ill-defined supramolecular structures (Fig. 2.3).

In the same context, the effect of composition, temperature, and pH has been also well-described by McCormick and coworkers in another case [8]. The authors studied the solution behavior of poly(N,N-diethylaminoethyl methacrylate)-b-poly(N-isopropyl acrylamide) (PDEAEMA-b-PNIPAM). This copolymer has a PDEAEMA pH-responsive block and a PNIPAM temperature responsive block. Therefore, macromolecular chains self-assemble into PDEAEMA-core/PNIPAM-shell spherical micelles at temperatures below the lower critical solution temperature of PNIPAM and at solution pH values greater than the pK_a of PDEAEMA. At the same time, by decreasing the pH to values lower than 7.5 and by increasing the temperature to values higher than 42 °C, the reverse structure is observed, i.e., spherical micelles with hydrophobic PNIPAM cores stabilized by a hydrophilic PDEAEMA shell. Interestingly, in the case of polymers with increased PNIPAM volume fraction, the above-mentioned reversal of structure leads to vesicles for temperatures higher than 38 °C. The aforementioned behavior, where a block copolymer can form micelles with either the first or the second block located in the core, depending on the external stimuli, is generally termed as “schizophrenic.”

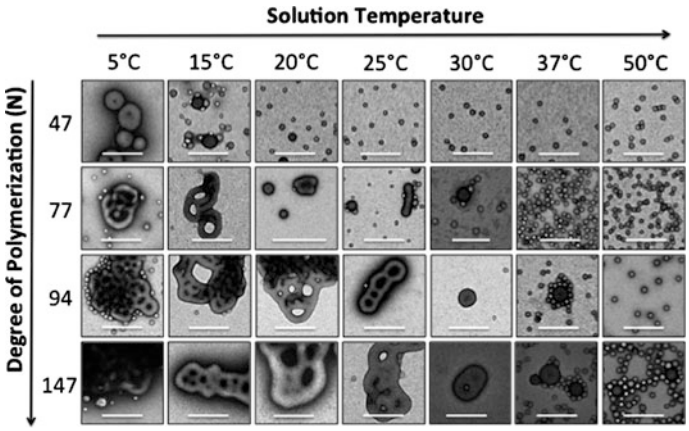

Fig. 2.3 The effect of temperature and composition of PMPC-b-PDPA on the formed structure at certain pH. Reproduced from [7] with permission from American Chemical Society

The formation of vesicles by block copolymers (also called polymersomes) is an interesting field of research due to their selectivity and bimodality in loading capacity (i.e., substances can be loaded within the polymersomes' outer layers or within the solvent pool in the center of the structures). An interesting example of a vesicle, formed by the triblock poly(N,N-diethylaminoethyl methacrylate)-b-poly(ε-caprolactone)-b-poly(N-isopropyl acrylamide) (PNIPAM-b-PCL-b-PDMAEMA) has been demonstrated by Liu et al. [9]. The polymer forms vesicles because of the presence of the PCL hydrophobic block. However, by increasing the temperature to 40 °C, the vesicle transforms to a spherical micelle with PCL and PNIPAM core and PDMAEMA corona. The transformation process seems to be reversible, since decreasing of temperature leads again to vesicles. Interestingly, the physicochemical characteristics of the vesicles respond to the presence of carbon dioxide. Therefore, under the stimulation of CO_2, the vesicular assemblies swelled while they adopted their initial characteristics upon purging with nitrogen.

The effect of CO_2 on the structural characteristics of polymer aggregates has been also demonstrated by Wang et al. [10]. In this case, a diblock copolymer containing one random copolymer block, namely the poly(ethylene oxide)-b-poly (4-vinyl pyridine-r-dimethyamino ethyl methacrylate) copolymer [PEO-b-P (4VP-r-DEAEMA)] was studied in aqueous media. It was found that the copolymer can self-assemble into vesicles in aqueous media and physiological pH, while the vesicles can be fused hierarchically into giant worm-like micelles of several micrometers in length. After bubbling CO_2 into the copolymer solution up to saturation, i.e., pH was changed to 5.43, the giant worms transformed again into large polymersomes. Notably, direct dissolution of the copolymer in acidic environment leads to small spherical micelles. Additionally, the vesicles obtained, after CO_2 saturation, could revert to worm-like aggregates after depleting CO_2. The above-described transition phenomena are mainly attributed to

protonation/deprotonation of the PDEAEMA units, to the strong steric hindrance effect from the adjacent 4VP groups and to hydrogen bonding between different 4VP units and free H_2O in the interior of vesicles.

Nanostructures of Non-common Morphologies

The characteristic examples, which are given above, outline some micellar systems with the so-called common structure. However, the focus in this section will be on the formation of non-common morphologies. Initially, an example which is located at the border line between common and non-common nanostructures is given below. In particular, the hierarchical self-assembly of an amphiphilic block copolymer, poly(N,N-dimethylacrylamide)-block-polystyrene with a very short hydrophilic block (PDMA-b-PS), into large granular nanoparticles has been described by Bianchi et al. [11]. The block copolymer forms water-soluble spherical micelles, however, the corona of these micelles has a rather unusual granular shape. The reason for this shape is the partial hydrolysis of the PDMA block.

Similarly, Lodge and coworkers have presented the formation of vesicles. However, these vesicles are not really common, since they incorporate hexagonally packed cylinders [12]. The aggregates were formed by the self-assembly of a miktoarm star terpolymer, where one of the arms is fluorinated. Interestingly, it was found that the assembly of these vesicles proceeds via the formation of metastable polygonal, faceted bilayer sheets.

Located at the common/non-common morphologies border, is also the nanostructure that has been presented by Hu and Liu [13]. Even though the formation of polymeric nanocapsules has already been intensively studied, the formation of polymeric nanocapsules bearing regularly sized nanochannels is a new observation. The use of a pseudo miktoarm triblock copolymer, namely μ-poly(tert-butyl acrylate)-poly(2-cinnamoyloxyethyl methacrylate)-poly(ethylene oxide) (μ-PtBA-PCEMA-PEO) can give polymeric nanocapsules in a mixture of water/THF, where the PEO is extended to the solvent, stabilizing the capsule, PCEMA forms the capsules and PtBA forms cylinders that permeated the PCEMA wall. Following photo-cross-linking of the PCEMA wall and hydrolysis of the PtBA blocks in the cylindrical domains yield unprecedented capsules bearing regularly packed uniform poly(acrylic acid)-gated nanochannels (Fig. 2.4).

The same principle, i.e., the formation of a nanostructure, using a terpolymer, followed by selective chemical modification of one of the blocks was also followed by Zhang et al. [14]. In this case, a triblock terpolymer, consisting of one hydrophilic block and two mutually incompatible hydrophobic blocks covalently connected by a redox-responsive disulfide linkage, self-assembled into multi compartment micelles, a type of micelles with subdivided hydrophobic cores, in aqueous solution. In particular, the micellar core is composed of two polymeric phases—one that is continued and a discontinued one. The formed nanostructure is

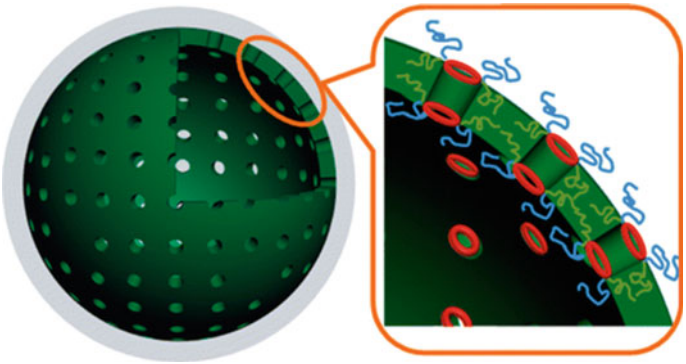

Fig. 2.4 Polymeric nanocapsules bearing regularly sized nanochannels. Reproduced from [13] with permission from American Chemical Society

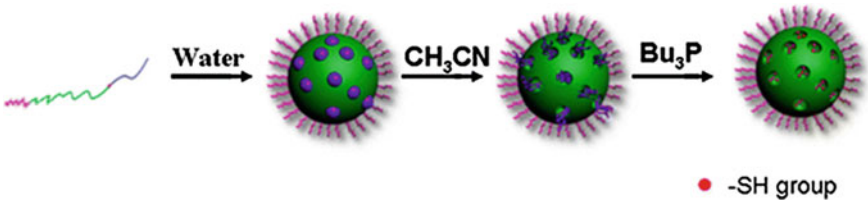

Fig. 2.5 Polymeric micelle with a mesoporous core using a block terpolymer. Reproduced from [14] with permission from American Chemical Society

subjected to cleavage of the disulfide linkage of the discontinued phase, leading to a polymeric micelle with a mesoporous core (Fig. 2.5).

The triblock copolymer poly(ethylene glycol)-b-poly(2-methyl-2-carboxyl-propylene carbonate)-b-poly(L-lactide) (mPEG-b-PMCC-b-PLA) has also been studied upon modification of the middle block with dopamine [15]. In this case, the polymer forms onion like micelles, i.e., micelles composed of a two layer core and a stabilizing shell, instead of the subdivided core that was described above. The onion like structure was subsequently stabilized by oxidative self-polymerization of dopamine at the middle block. The formed nanostructure was found to be superior, in comparison to the uncross-linked precursor, in drug loading. Generally, the cross-linking approach is frequently used in order to stabilize complex architectures or to improve the micellar functionality.

The concept of cross-linking has been used by Cohn and coworkers for the formation of temperature-responsive nanoshells [16]. The nanoshells are formed using the triblock poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) decorated with methacrylic groups at the two ends of the macromolecular chain. In particular, the triblocks form micellar structures at elevated temperature, where the methacrylate units are located at the outer phase of the shell. By performing a cross-linking reaction at the methacrylic units, the ends of

the PEO block of each micelle are connected together. Following, the temperature is decreased and the PPO core is dissolved, leading to a flexible nanoshell. Various parameters, like the reaction temperature, the polymer concentration, and the presence of triblocks without methacrylic terminal groups have been found to strongly affect the structural characteristics of the nanoshells. Interestingly, even tubular nanoshells were observed by performing the reaction at elevated temperature, where the transformation of the spherical micelles to those of rod-like shape is favored.

The cross-linking approach was used for the stabilization of micellar nanostructures which are formed by the block copolymer poly[oligo(ethyleneglycol) methacrylate]-block-[poly(styrene)-co-poly(vinyl benzaldehyde)] [POEGMA-b-P(ST-co-VBA)] [17]. The interesting feature of this polymer is that it changes the micellar structure that adopts by changing the degree of polymerization (DP) of the second block. Therefore, spherical micelles are formed from the block copolymer when DP of the P(ST-co-VBA) block reaches up to 175 units. A morphology transition from micelles to worm and rod-like structures was observed for P(ST-co-VBA) blocks greater than 340 repeating units. Finally, vesicles were formed when DP of the second block reached over 500. All the above nanostructures can be stabilized using a diamine in order to cross-link the aldehyde groups.

Multicompartment Micelles

Even though cross-linking is a great tool toward the synthesis and stabilization of novel polymeric nanostructures, the advances in polymer chemistry have made possible the synthesis of macromolecules that tend to adopt some very sophisticated nanostructures without cross-linking. Multicompartment micelles are a very important class of polymeric non-common nanostructures. Generally, they are composed by a terpolymer, linear or with more complex architecture, where one of the blocks is soluble in the selective solvent, while the other two are insoluble to the solvent and, at the same time, they are strongly incompatible. Janus micelles, named after the roman god Janus who had two faces, is a typical example of multicompartment micelles. Wang et al. [18] have recently described a well-studied polymer that gives Janus micelles in aqueous solutions. The aforementioned polymer is of a A-block-B-graft-C architecture composed of biologically compatible polymers, methoxy poly(ethylene glycol) (PEG), poly(ε-caprolactone) (PCL) and poly(2-(perfluorobutyl)ethyl methacrylate) (PPFEMA). The PEG block is extended to the solution, stabilizing the nanostructure, while the PCL and PPFEMA blocks are located in the micellar core. However, the PCL segments are in crystalline state, while fluorocarbon segments are amorphous, leading to the formation of a Janus-core with adjustable compartment balance, as was revealed by complimentary techniques.

Another example of multicompartment micelles is the case where the solvophobic part of the micelle is composed by a core and some distinct nodules at the

surface of the core, while the micelle is stabilized by a solvophilic corona. In the case of the above structure, both terpolymers, like in the examples described above, and block copolymer mixtures have been used. The terpolymer poly ((sulfamate-carboxylate)isoprene)-block-polystyrene-block-poly(ethylene oxide), PISC-PS-PEO, can be considered as case study for the formation of multicompartment micelles with nodules [19]. In acidic solutions, the pH-responsive terpolymers self-assemble into kinetically trapped multicompartment micelles, where the micellar solvophobic part is consisting of discrete PS nodules and PISC core, while the PEO chains are located in the shell. Because of the kinetically frozen nature of the formation, the copolymer composition of low PS weight percent can be considered as a critical parameter. Notably, the aforementioned terpolymer forms regular spherical micelles with PS core and mixed PISC/PEO corona, in alkaline solution.

Finally, the in situ polymerization of poly(4-vinylpyridine) using a capping group located at the free corona chain ends of a polystyrene-b-poly(N, N-dimethylacrylamide) (PS-b-PDMA) spherical micelles with PS core, can also lead to multicompartment micelles [20]. The formed P4VP tend to aggregate on the PS surface, creating nodules. Depending on the polymerization degree of the P4VP the nodules can grow larger, forming, finally, concentric core–shell–corona nanoparticles. This is a typical example of how the composition of the terpolymer can lead to various shaped multicompartment nanostructures.

An extreme case of multicompartment nanostructure has been recently described by Shi et al. [21]. They have studied the case of a linear triblock copolymer where the two end blocks were poly(N-(2-methacryloyloxyethyl)pyrrolidone) (PNMEP), while the middle macromolecular chain was high density grafted poly(t-butyl acrylate)-b-polystyrene (PBA-b-PS). The aforementioned copolymer was found to form soft disk-like micelles by hexagonally packing of the middle block (Fig. 2.6). It has to be noted that the hexagonal pattern of the molecular brushes aligned perpendicularly to the disk plane, while this pattern demonstrates a periodic

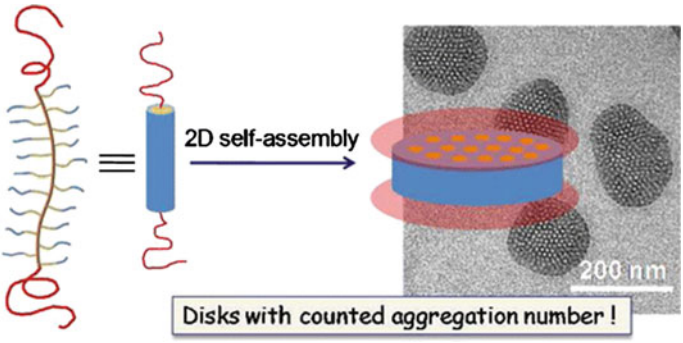

Fig. 2.6 Multicompartment nanostructure of soft disk-like micelles with perpendicular areas of hexagonally packing blocks. Reproduced from [21] with permission from American Chemical Society

spacing. The above example is indicative of the high structural complexity level that can be reached, as outcome of inspired macromolecular architectures.

Hybrid Nanostructures

Staying in the framework of multicompartment micelles, one should note that the formation of this nanostructure can be also achieved by a mixture of two block copolymers. Zhang and coworkers have described the case of a thermoresponsive multicompartment micelle composed of two block copolymers, namely poly[N-(4-vinylbenzyl)-N,N-diethylamine)]-b-polystyrene (PVEA-b-PS) and poly[2-(dimethylamino) ethyl methacrylate]-b-polystyrene (PDMAEMA-b-PS) [22]. A mixture of these copolymers, in a polar solvent, forms micelles where the PS core is decorated with PVEA nodules (Fig. 2.7). Depending on the solvent, the multi-compartment nanostructure can be either precipitated at elevated temperature, when water is used as solvent, or to be reformed into a regular micelle with PS core and mixed PVEA/PDMAEMA corona, when the solvent is methanol, just as it was described in the case of the pH-responsive terpolymer [19].

Going beyond the incompatibility of polymer chains with solvents or/and other polymers, that has been described above, the crystallization of a macromolecule can be potentially the driving force toward the formation of nanostructures. An interesting example is the case of a mixed system composed of PEO-b-PCL and PCL homopolymer [23]. In this system, the formation of nanorafts is recorded through a novel mechanism of lamella formation, involving the 2D alignment of block copolymer rod micelles. In particular, PCL chains tend to crystallize in spherical shape. The sphere-type aggregation provides the building blocks for growth along an orthogonal direction into rods. Finally, the rods align to the final 2D nanoraft product. Interestingly, the size and the aspect ratio of the formed nanostructures are highly controllable by small changes of the homopolymer content in the mixture.

Continuing in the context of mixed systems, i.e., systems composed of a block copolymer and one (or more) other entity, Betthausen et al. [24] has described a

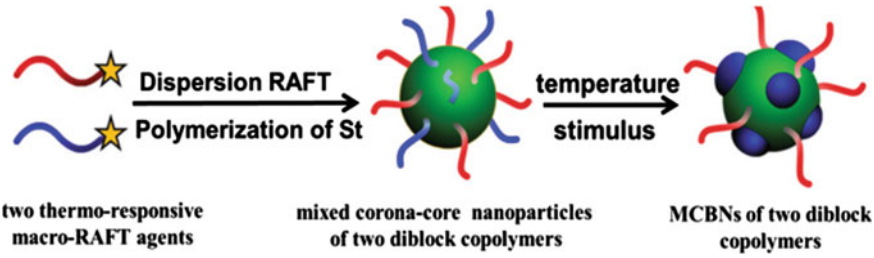

Fig. 2.7 Multicompartment micelles of PS core, decorated with PVEA nodules. Reproduced from [22] with permission from Royal Society of Chemistry

plethora of nanostructures that can be adopted by a linear ABC triblock terpolymer comprising a polyamine block: polybutadiene-b-poly(tert-butyl methacrylate)-b-poly(2-(dimethylamino)ethyl methacrylate) (PB-PtBMA-PDMAEMA) in the presence of organic di- or triacids, in mixtures of THF and water. The interaction of the PDMAEMA block with the organic acids is the driving force for the formation of nanostructures. A number of parameters, like chain architecture, amount, and functionality of added acid, the solvent quality, and the PDMAEMA block length, seem to influence the shape of the aggregates finally formed. Depending on the balance among the above parameters, a variety of structures is observed, such as spherical, disk-shaped, toroidal, ribbons featuring enlarged end-caps structures, and alongside undulated ribbons.

An even higher level of structural complexity can be achieved using binary mixtures of block copolymers. The simple solution construction of multigeometry nanoparticles, disk-sphere and disk-cylinder, through a straightforward, molecular-level blending of block copolymers has been described in an inspired work of Zhu et al. [25]. The multigeometry nanoparticles contain disk geometry in the core with either spherical patches along the disk periphery, in the case of disk-sphere particles, or cylindrical edges and handles in the case of the disk-cylinder particles. It has to be noted that the formation of the above extraordinary nanostructures is dictated not only by thermodynamic parameters, i.e., interactions between the polymers, but also by kinetic of aggregation.

Nanostructures via Electrostatic Interactions

Another possibility for obtaining multicompartment micelles, in the framework of mixed systems, is the case of electrostatic complexation. Synatschke et al. [26] have presented a system where the formation of micellar interpolyelectrolyte complexes (IPEC) leads to multicompartment micelles. In particular, the triblock polybutadiene-b-poly(1-methyl-2-vinylpyridinium methylsulfate)-b-poly(methacrylic acid) complexes with a high charge density cationic polyelectrolyte, provides a compartmentalized IPEC shell (Fig. 2.8). It was found that the complexes are not kinetically frozen and that the nature of the cationic moiety plays a minor role to the compartmentalization procedure. In contrast, the length of the anionic block, belonging to the triblock, seems to be the key factor for the compartmentalization, since the effect is more pronounced when the poly(methacrylic acid) block is longer.

The electrostatic interactions are not used frequently in the spatially ordered nanostructures, mainly due to their isotropic nature. However, like the above-mentioned example, there are some noteworthy characteristic cases. The formation of an ultralong nanoladder, composed of a block copolymer and a stiff bisligand has been described by Xu et al. [27]. In particular, the block copolymer composed by a cationic and a neutral block, interacts with the anionic stiff

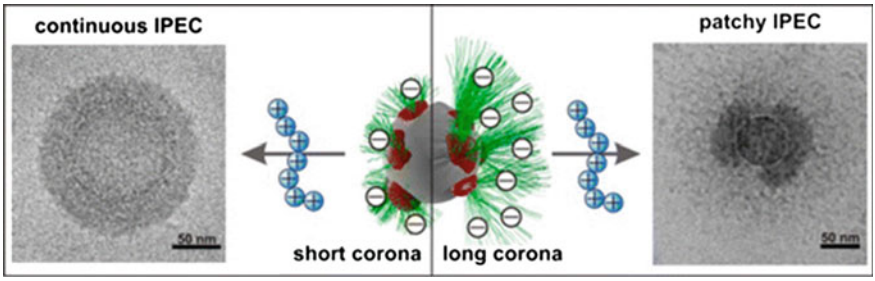

Fig. 2.8 Multicompartment micelles, assisted by electrostatic complexation. Reproduced from [26] with permission from American Chemical Society

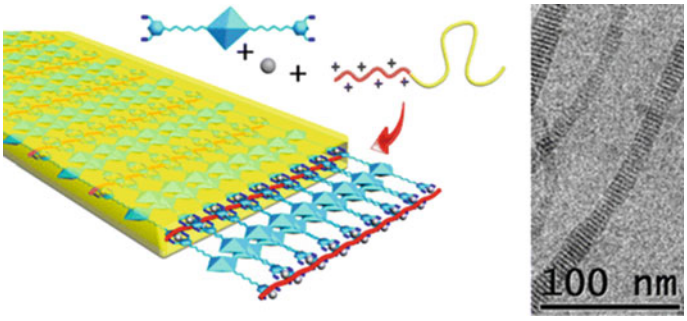

Fig. 2.9 Formation of an ultralong nanolander, composed of a block copolymer and a stiff bisligand. Reproduced from [27] with permission from American Chemical Society

bisligand, where the anionic sites are located at the two ends of the ligand. The result is nanoladders, enclosed in a matrix of the neutral block (Fig. 2.9).

The electrostatic interactions are important also in the class of polyelectrolyte aggregates. An interesting example is the case of the star block copolymer $PS_n(P2VP\text{-}b\text{-}PAA)_n$ [28]. In this case, the PS arms are always collapsed in aqueous environment, while the P2VP and PAA are positively and negatively charged, respectively, depending on the solution pH. Therefore, the electrostatic interactions between the charged moieties, in addition to the complex architecture of the star block copolymer, can lead to interesting transformations of the aggregate structures upon changes of the solution pH. Hence, at pH 1.4 unimolecular micelle are observed, which are transformed to multicore micelle (pH 1.6) and to worm-like micelles (pH 2). It is noteworthy that these transformations take place in a very narrow range of solution pH. On the other hand, in basic conditions, unimolecular micelle and network-like assembly are recorded at pH 8.5, while increasing pH to 11.8, the formation of multicompartment micelles is observed.

A whole new approach, toward the formation of complex structures by simple block copolymers, using electrostatic interactions with divalent counter ions, has been presented by Cui et al. [29]. They have proposed a technique that relies on

divalent organic counter ions and solvent mixtures in order to drive the organization of the block copolymers down specific pathways into complex one-dimensional structures. In particular, using a triblock terpolymer, PAA-b-PMA-b-PS, and an organic diamine, in a proper mixture of solvents, they managed to formulate aggregates with a segmented cylinders morphology.

Hierarchical Self-assembly

Beyond the concept of multicompartment micelles, an important class of non-common polymeric nanostructures is that of nanoparticles obtained by hierarchical self-assembly. In this case the block copolymers form some initial assemblies, which, in most of the cases, are simple structures. Then, the aforementioned assemblies aggregate toward the formation of larger structures. In a third step, the aggregates can "collaborate together" in order to create a more complex nanostructure and so on. A characteristic example of these hierarchically self-assembly nanoparticles is the case of the fully conjugated poly(3-(2-ethylhexyl) thiophene)-b-polythiophene (P3EHT-PT). This block copolymer forms nanospheres which are coming together forming nanorods, which, in their turn, are aggregated in order to create nanostars, which are connected together for the formation of nanonetworks as schematically presented in Fig. 2.10 [30].

One of the most characteristic cases of hierarchically self-assembled nanoparticles is that presented by Muller and coworkers on the solution behavior of a linear

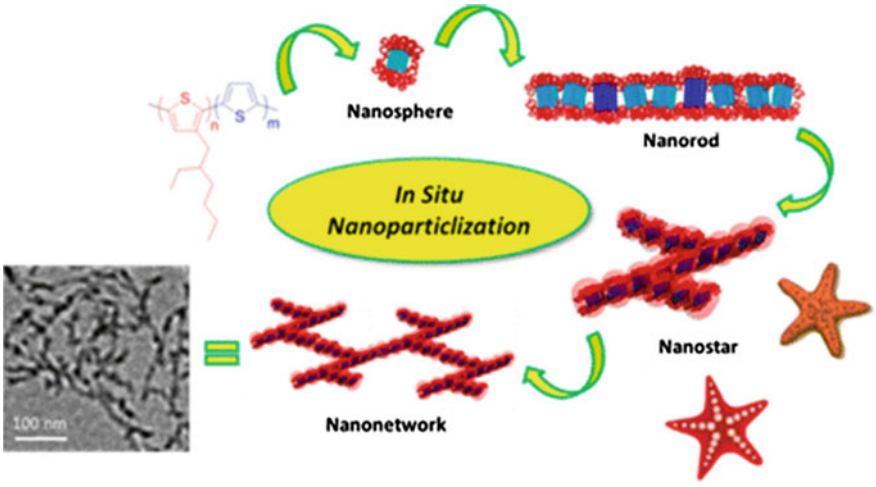

Fig. 2.10 Hierarchical self-assembly nanoparticles of a block copolymer that forms nanospheres which are coming together forming nanorods, which are aggregated to nanostars, which are connected together for the formation of a nanonetwork. Reproduced from [30] with permission from American Chemical Society

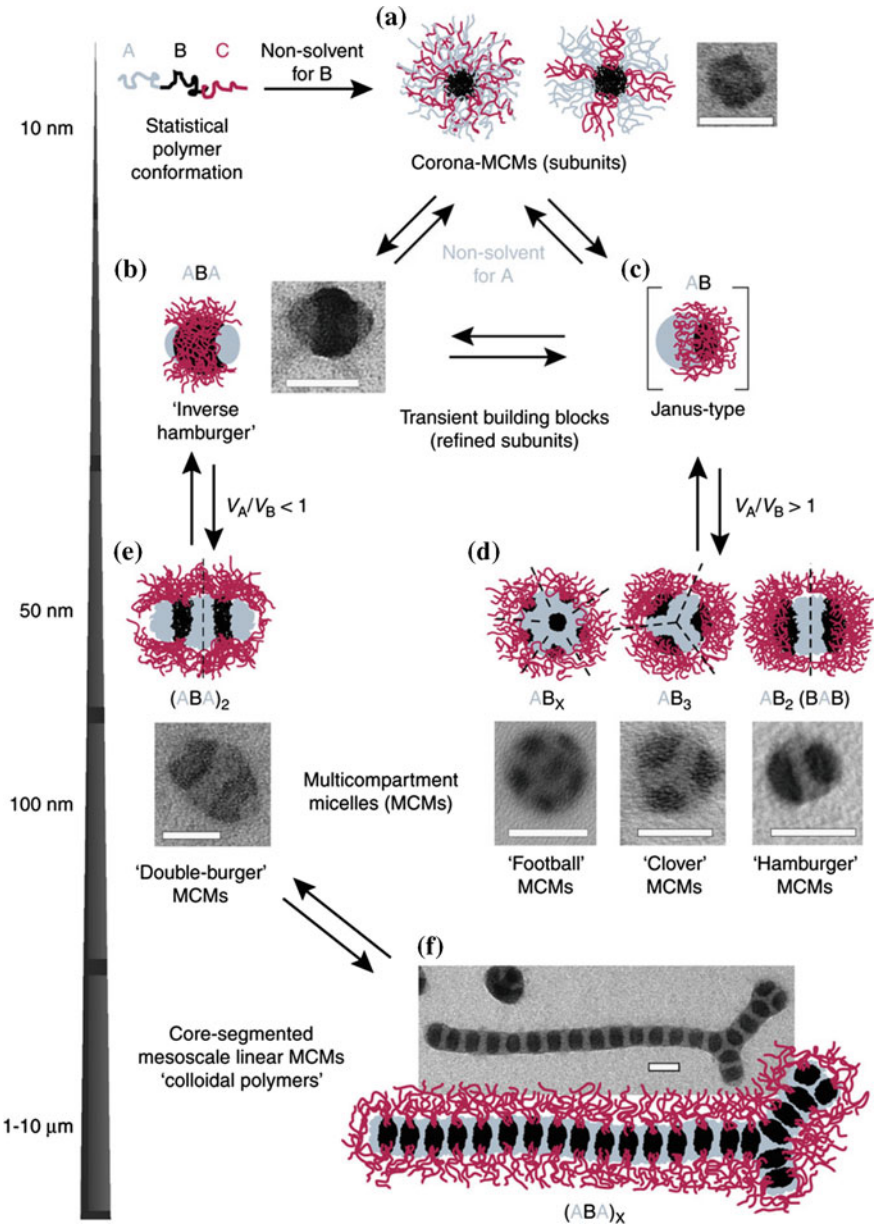

Fig. 2.11 Hierarchical self-assembly of a linear triblock copolymer. Reproduced from [31] with permission from Nature Publishing Group

triblock copolymer [31]. The used copolymer, namely PS-PB-PMMA, initially forms well-defined spherical micelles with mixed PS/PMMA corona, in a thermodynamically bad solvent only for the PB block (Fig. 2.11). The hierarchical self-assembly takes place when the micelles are dialyzed against a non-solvent for

both the PS and PB blocks causing the collapse of PS blocks. This collapse can be done in a way that Janus-type or "inverse-burger" micelles can be formed. In a second degree of organization, the formation of a number of distinct multicompartment micelles, depending on the PS/PB ratio, like aggregates that resemble to double burger, soccer ball, clover or hamburger can be formed. Finally, in the last hierarchically step, the micelles link together for the formation of elongated structures of PS, covered by PMMA and doped with PB spheres. All stages of organizations are presented in Fig. 2.11.

Nanostructures Formed on a Surface

A large number of works deal with the structures formed by micelles of molecularly dissolved block copolymers when they are drying on a solid surface under appropriate conditions. Herein, only few interesting examples will be discussed, in an effort to outline this area of research. Perfluorinated polymers are frequently used for the formation of these dry nanostructures, due to their unique physical characteristics. A characteristic case is that of poly(1,3-(4-formyl-phenoxy)-2-hydroxypropyl methacrylate)-b-poly(poly(ethylene glycol) methacrylate)-b-poly (2,2,3,4,4,4-hexafluorobutyl methacrylate) (PFPHPMA-b-PPEGMA-b-PHFBMA) terpolymer [32]. The particular block terpolymer forms botryoid shaped nanostructures under appropriate drying conditions. In particular, PFPHPMA domains are miniaturized into small-sized discrete "grapes" and attached onto the outwardly branched scaffolds of fluorinated segments. Such kind of nanostructure is appealing as sensor or in catalytic applications, due to their increased accessible surface.

Another interesting example is the formation of Janus particles. Mueller and coworkers, since 2003, have demonstrated the formation of Janus cylinders using a carefully designed triblock copolymer and bulk cross-linking procedures [33]. In the same concept, the same group has also recently demonstrated that by controlling the phase transitions via pretreatment and cross-linking conditions of the lamella–cylinder equilibrium bulk morphology of a block copolymer, namely poly (tert-butoxystyrene)-b-polybutadiene-b-poly(tert-butyl methacrylate), the formation of Janus cylinders, sheets, or even ribbons can be observed [34]. The key factor toward the formation of the above mentioned structures is the fine control of the parameters, before and during drying, in order to take the appropriate bulk structure.

Finally, Petzetakis et al. have described a facile protocol for the production of nanocages and nanotubes on a surface by simple drying of a diblock copolymer solution. In particular, they have studied the case of poly(acrylic acid)-b-polylactide (PAA-PLA). This copolymer forms spherical and cylindrical micelles. However, these micelles tend to spontaneously form hollow nanocages and nanotubes upon evaporation of the solvent [35]. Additionally, the internal topology of the PAA-PLA particles could be tuned by manipulating the drying conditions to give solid or compartmentalized structures. Interestingly, upon resuspension, these

reorganized nanoparticles retain their hollow structure, making the system, and the whole procedure, appealing for a number of applications.

Polymerization-Induced Self-assembly

As already described in the previous sections, the classical approach for preparation of polymeric nano-size aggregates from amphiphilic block copolymers by self-assembly in a selective solvent involves several steps. The first step is synthesis of an amphiphilic copolymer with a narrow molar mass distribution, followed by purification and characterization. Then, the self-assembly is performed by adding a non-solvent of one block into dilute copolymer solution or by direct dissolution of the copolymer in a selective solvent. Usually, the copolymer concentration varies between 1 and 10 g L^{-1} (0.1–1 wt%).

Recently, another feasible method, called *polymerization-induced self-assembly* (PISA), has been introduced for preparation of block copolymer nanoobjects with relatively high copolymer concentration (10–30 wt%). This is "one-pot" approach where the formation of block copolymers, self-assembly, and morphology transition are accomplished in the same polymerization system [36]. In general, the polymerization media is a good solvent for the first block and a non-solvent of the second block. Thus, during the chain growth of the second block, at certain degree of polymerization (critical micelle degree of polymerization, CMDP) it becomes insoluble and triggers microphase separation of the system. In other words, the block copolymers form aggregates in situ during the chain growth of the second block (Fig. 2.12).

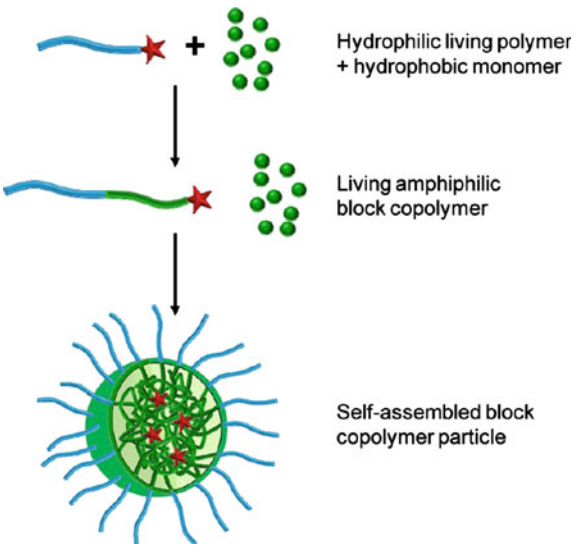

Fig. 2.12 General principle of the polymerization-induced self-assembly method. Reproduced from [36] with permission from Royal Society of Chemistry

According to the solubility of the core-forming monomer in the reaction media, two different methods—emulsion polymerization and dispersion polymerization—have been exploited to obtain self-assembled nanoparticles by PISA [37, 38]. The dispersion polymerization can be carried out either in water or in organic solvents. The emulsion polymerization starts from a monomer-in-water emulsion, where a water-soluble polymer precursor is chain-extended by polymerizing a water-immiscible monomer, resulting in self-assembled block copolymers. In contrast to the emulsion polymerization, dispersion polymerization is conceptually much simpler and the initial reaction solution is homogeneous.

Since the self-assembly phenomena requires well-defined block copolymers with narrow molecular weight distribution, only living/controlled polymerization techniques have been exploited for the preparation of polymeric nanoparticles via PISA. In particular, in aqueous media, the controlled radical polymerizations have been the methods of choice, taking the advantage of the compatibility of the reactions with water and the ability to create a wide variety of amphiphilic polymers. Atom transfer radical polymerization (ATRP), nitroxide-mediated polymerization (NMP), and reversible addition-fragmentation chain transfer (RAFT) polymerization have been the most studied techniques. The three methods possess certain advantages; however, RAFT remains particularly the most attractive due to the wide variety of polymers that can be produced in a controlled manner at low polymerization temperatures.

Ferguson, Hawkett and coworkers have pioneered the field of polymerization-induced self-assembly by employing RAFT emulsion polymerization of acrylic acid and n-butyl acrylate in aqueous media [39]. This approach led to in situ self-assembled nanoparticles formed by an amphiphilic block copolymer. First, acrylic acid was polymerized in the water phase to yield a water-soluble macro-RAFT agent, followed by the polymerization of hydrophobic n-butyl acrylate. At the completion of the polymerization, self-stabilized micelle-like PAA-b-PnBA particles of number-average diameter of 60.3 nm were obtained. When polymerization was continued by feeding of another hydrophobic monomer, styrene, spherical core-shell particles composed of poly(acrylic acid)-b-poly(butyl acrylate)-b-polystyrene triblock copolymer were formed [40].

Charleux and coworkers have made considerable progress toward the understanding of self-assembly phenomena of nanoparticles obtained via aqueous emulsion polymerization. Thus, poly(ethylene oxide) and poly(N, N-dimethylacrylamide)-based water-soluble macro-RAFT agents were chain-extended by polymerizing a water-immiscible monomer such as styrene, methyl methacrylate, or n-butyl acrylate in a batch emulsion polymerization [41–43]. Starting with the idea that the synthesized amphiphilic block copolymers would simply stabilize classical latex particles, it was realized that the good control of the polymerization of the water-immiscible monomer allowed in situ formation of well-defined spherical self-assembled polymeric nanoparticles (Fig. 2.13). It was suggested that only when the degree of polymerization of the hydrophobic block is large enough, the so-formed amphiphilic block copolymer chains can self-assemble into micelles.

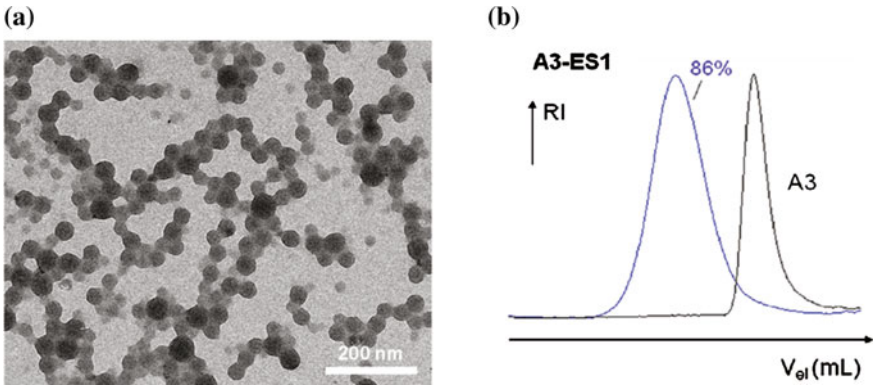

Fig. 2.13 **a** TEM image for a sample prepared by the emulsion polymerization of styrene with PDMAAm macro-RAFT agent; **b** SEC chromatograms in THF of the polymer from the same experiment. Reproduced from [43] with permission from American Chemical Society

Aqueous dispersion polymerization is another approach studied for in situ preparation of self-assembled amphiphilic block copolymer nanoparticles. In contrast to the aqueous emulsion polymerization, an important prerequisite in dispersion polymerization is the selection of a water-soluble monomer which, when polymerized, forms a water-insoluble polymer. First, An and coworkers reported on RAFT aqueous dispersion polymerization of N-isopropylacrylamide (NIPAAm) initiated with poly(N,N′-dimethylacrylamide)-based (PDMAAm) macro-RAFT agent [44]. With the progress of polymerization, an amphiphilic PDMAAm-b-PNIPAAm block copolymer was formed, and it further self-assembled into block copolymer micelles when PNIPAAm blocks became sufficiently long to collapse at the reaction temperature of 70 °C. The use of cross-linking agent was necessary to avoid dissociating of the micelles at room temperature. In the same year, Charleux and coworkers published a study of similar system with the core-forming block based on poly(N,N-diethylacrylamide) instead of NIPAAm using nitroxide-mediated polymerization [45]. Later on, Li and Armes [46] reported another aqueous dispersion polymerization formulation for in situ syntheses of core-shell nanoparticles. A poly(glycerol monomethacrylate)-based macro-RAFT agent was first synthesized and then used to initiate the propagation of the water-soluble monomer 2-hydroxypropyl methacrylate (HPMA). Since the corresponding polymer, PHPMA, is water insoluble, the microphase separation occurred when the chain length of PHPMA reached CMDP. It was demonstrated that the size of the formed nanoparticles can be controlled by varying the length of PHPMA chains. Further studies of the same group revealed that not only spherical micelles could be formed by PISA in aqueous media [47]. For example, the chain extension of highly hydrated zwitterionic poly(2-(methacryloyloxy) ethylphosphorylcholine) (PMPC) block with HPMA in water at 70 °C produced a hydrophobic PHPMA block, which triggered in situ self-assembly to form well-defined diblock copolymer spheres, worms, or vesicles. The final particle

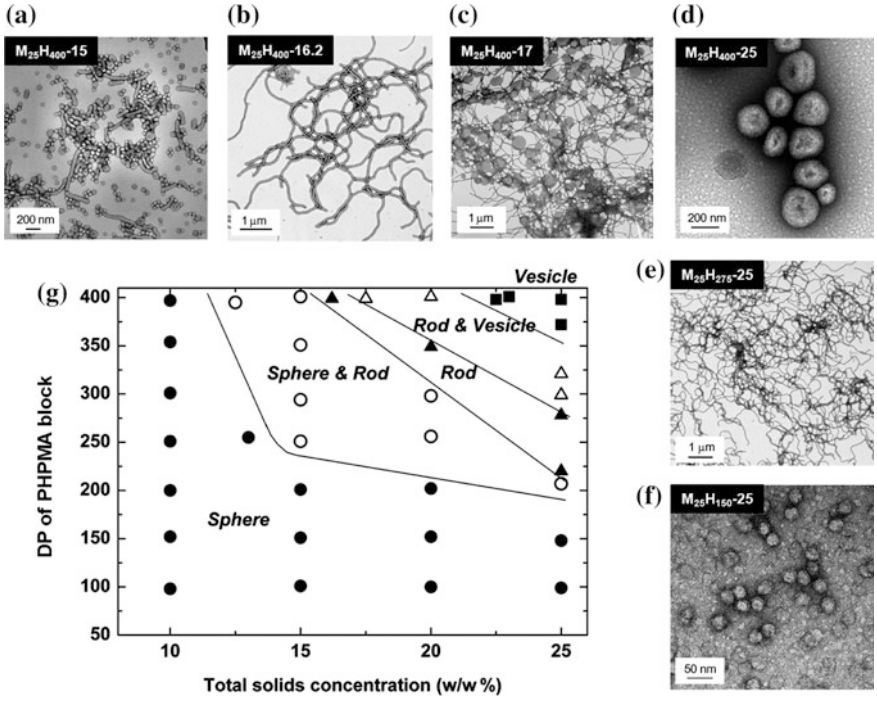

Fig. 2.14 Detailed phase diagram constructed for the $M_{25}H_x$-Y formulation (where M denotes MPC and H denotes HPMA) by systematic variation of the mean target degree of polymerization of PHPMA (x) and the total solids concentration (Y) used for each synthesis. The mean DP values of the PHPMA block shown in the phase diagram were calculated from the diblock copolymer composition determined by ^{1}H NMR spectroscopy in d_4-methanol assuming 100 % blocking efficiency for the $PMPC_{25}$ macro-CTA. TEM images for representative morphologies: **a** $M_{25}H_{400}$-15 (spheres and worms), **b** $M_{25}H_{400}$-16.2 (worms), **c** $M_{25}H_{400}$-17 (worms and vesicles), and **d** $M_{25}H_{400}$-25 (vesicles), i.e., identical diblock copolymers prepared at differing copolymer concentrations. **e** $M_{25}H_{275}$-25 and **f** $M_{25}H_{150}$-25 are two other diblock copolymers prepared at the same 25 wt% solids content used for image **d**. Reproduced from [47] with permission from American Chemical Society

morphology obtained at full monomer conversion is dictated by the degree of polymerization of the PHPMA block and the total solids concentration at which the HPMA polymerization is conducted. It is found that the onset of micellar nucleation corresponds to an enhancement in the rate of polymerization, which suggests solvation of the growing PHPMA chains by the unreacted HPMA monomer. Moreover, close monitoring of the in situ HPMA polymerization by TEM revealed a range of intermediate morphologies, which provide important information regarding the mechanism of sphere-to-worm and worm-to-vesicle transitions (Figs. 2.14 and 2.15) [47].

Pan and coworkers have published a series of papers describing the formation of spherical and worm-like micelles, vesicles, nanotubes, and some other morphologies

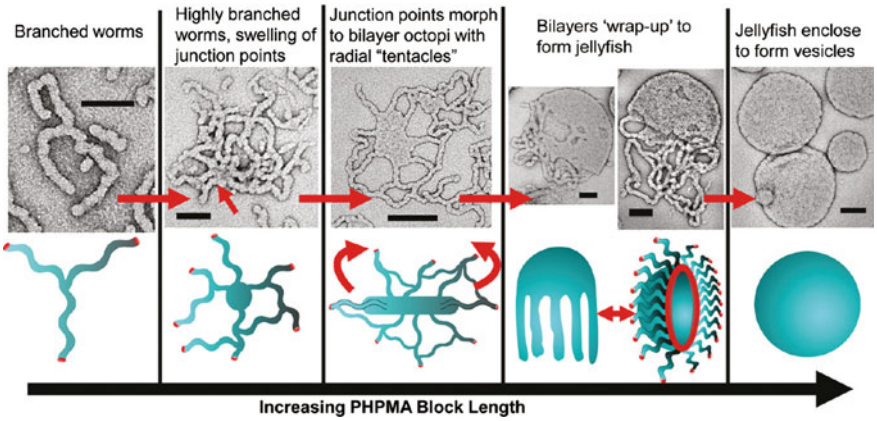

Fig. 2.15 Suggested mechanism for the polymerization-induced worm-to-vesicle transformation during the synthesis of G47-H200 by RAFT aqueous dispersion polymerization. Reproduced from [47] with permission from American Chemical Society

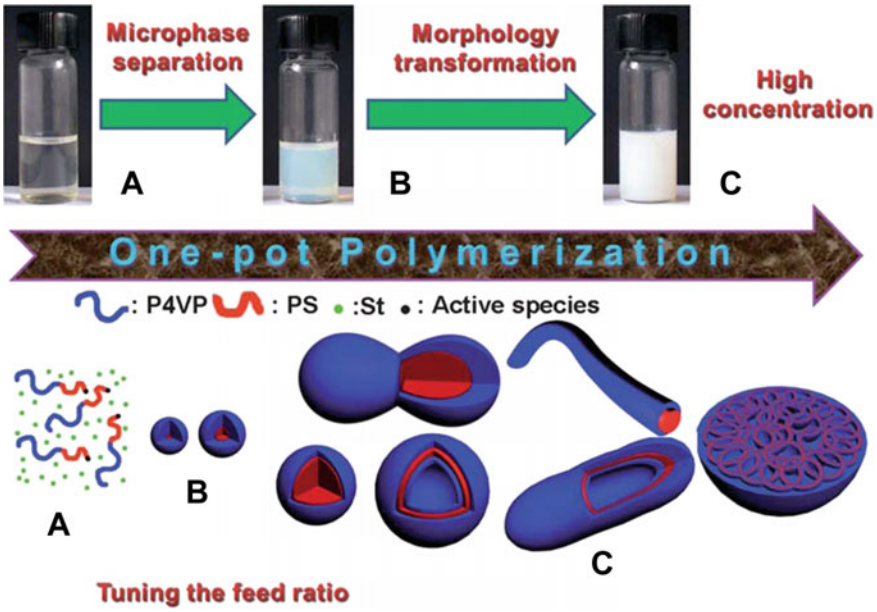

Fig. 2.16 The formation mechanism of the multiple morphologies in the RAFT polymerization of S in methanol using P4VP-TC as macro-RAFT agent, (*A*) formation of the soluble block copolymer, PS-b-P4VP; (*B*) phase separation to form spherical micelles; (*C*) re-organization of the resulting spheres to yield multiple morphologies. Reproduced from [48] with permission from Royal Society of Chemistry

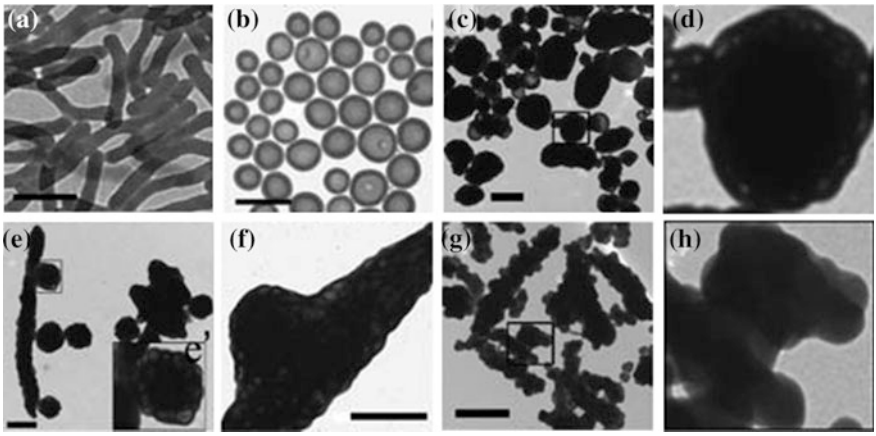

Fig. 2.17 TEM images of the aggregates prepared by RAFT polymerization of S at 80 °C for 36 h with different amounts of methanol: **a** 1.5 g; **b** 1.2 g; **c** and **d** 0.9 g; **e** and **f** 0.8 g; **g** and **h** 0.5 g. **d**, **e′** and **h** are magnified images of the parts in the square frames of **c**, **e** and **g**, respectively. Feed molar ratio: PDMAEMA/S/AIBN = 10:250,000:1, S:2.0 g. The scale bars are 500 nm (**a**, **b**) and 1 mm (**c**, **e**, **f**, **g**), respectively. Reproduced from [51] with permission from Royal Society of Chemistry

via RAFT dispersion polymerization in methanol [48, 49]. Typically, the synthesized block copolymers comprised polystyrene as the core-forming and structure-directing block. In the reversible addition-fragmentation chain transfer polymerization of styrene in methanol using poly(4-vinylpyridine) as a macro-RAFT agent (P4VP-TC), due to continuous alteration of the solvophobic to solvophilic block balance, two phase transitions occurred—phase separation to form spherical micelles and reorganization of the resulting spheres to yield multiple morphologies including nanorods, nanotubes, vesicles, and doughnuts. Initially, S, P4VP-TC and the initiator were soluble in methanol and the polymerization proceeded homogeneously (Fig. 2.16A). Since PS is insoluble in methanol, when the PS blocks grow to a critical value, phase separation occurs to form spherical micelles (Fig. 2.16B). To transfer the formed spherical micelles into other nanostructures, one key point is the continuous growth of the core-forming chains in the core–shell particles. Since the solubility parameter (d) of methanol (d = 29.7) is much higher than that of PS (d = 16.6–20.3), improving the solubility of the polymerization media with respect to the PS core is necessary for maintaining the propagation rate of PS chains in the cores of micelles. Consequently, the addition of a higher content of S (d = 19.0) in the feed acts as co-solvent before its polymerization [50]. The increase of PS chain length resulted in an increase of the packing parameter, leading to curvature decreasing of the polymeric assemblies. As a result, the spherical micelles were transferred to other morphologies along with a decrease of curvature as shown in Fig. 2.16C. Noteworthy, at high monomer conversion the morphology formed by phase separation was generally locked because the polymerization temperature was below the glass transition temperature of PS. Different morphologies can also be obtained by changing the relative content of methanol in the

polymerization system [51]. TEM analysis confirmed that the RAFT polymerization of S, using a PDMAEMA-based macro-RAFT agent, at different weight ratios of methanol to S resulted in various morphologies. With the decrease of methanol content, the final structures vary from nanostrings (Fig. 2.17a) to spherical vesicles (Fig. 2.17b), to large compound vesicles (Fig. 2.17c) and to other ill-defined aggregates (Fig. 2.17e, g).

Biomedical Applications

Self-assembled block copolymers nanostructures could be particularly interesting toward biomedical applications, mainly due to their amphiphilic nature. They can serve as nanocarriers and/or stimuli to the formation of sophisticated nanostructures incorporating pharmaceutical compounds. A large number of publications deal with the potential use of block copolymers as drug carriers. Typically, a drug molecule interacts with a block copolymer, through either electrostatic or hydrophoblic interactions (depending on the nature of the drug). The polymer-drug nanoassembly is protected by the one of the blocks, typically PEO chains. Moreover, molecules and/or nanoparticles that permit the release of the drug in a specific area are generally used in order to reduce the drug toxicity.

The electrostatic complexation is widely used toward this end, because of the reversible nature of this complexation, due to changes in salinity or pH. Moreover, the complexation of an oppositely charged drug molecule and polymer chain leads to the formation of a water-insoluble polyion complex which stays in solution because of the second hydrophilic block. Following this concept, Li et al. [52] reported the complexation of the cationic drugs dibucaine, tetracaine, and procaine with anionic poly (methacrylic acid)-b-poly(ethylene oxide) (PMA-b-PEO) copolymers, which leads to micelles where the ionic drugs are located in the micellar cores. The study of the above micelles leads to the conclusion that both the electrostatic interactions and the hydrophobic interactions play an important role to the properties of the obtained micelles. In the same concept, triblocks can be also used as drug carriers, such as poly (ethylene oxide)-b-poly[sodium 2-(acrylamido)-2-methyl-1-propanesulfonate]-b-polystyrene (PEO-b-PAMPS-b-PS) triblock terpolymer [53]. Spherical micelles with zero surface charge and a stealth PEO corona were formed while the amount of the incorporated drug is controlled by the length of the charged block (PAMPS). It has to be noted that the charge ratio, [+]/[−], plays a key role on the physicochemical characteristics of the micellar complexes, as was studied by Soliman and Winnik [54]. In particular, the formation of micelles, their size and stability as well as drug uptake and release are characteristics connected to the above-mentioned ratio.

Besides the self-assembled spherical micelles, other polymeric nanostructures have been also used as drug carriers. Vesicles are of particular interest, since they can incorporate either hydrophobic drugs (in the hydrophobic polymeric part of the outer membrane), or hydrophilic drugs (inside the inner cavity). Xu et al. have presented the case of a linear triblock terpolymer, namely poly(ethylene oxide)-b-poly(acrylic acid)-b-poly(N-isopropyl acrylamide) (PEO-PAA-PNIPAM). This

copolymer is molecularly dissolved at room temperature, while it forms nano-sized vesicles upon increasing the solution temperature. The formed nanostructures are stabilized in the vesicle form by cross-linking of the PAA block. The formed stabilized vesicles could be used as carriers of molecules with bioactivity, offering quite increased loading efficiency. Due to the stabilized nature of the carrier, the nanostructure is resistant to temperature and salinity changes, as well as to dilution (a very important parameter for a system that is supposed to be inserted in the systemic circulation). Even more, the carrier seems to dissociate under reductive conditions (similar to that observed at intracellural areas), making the system ideal for intracellural drug delivery [55]. Beyond vesicles, other cross-linked polymeric nanostructures have been also reported in the literature, based on the same concept as before. A characteristic example is the case of a triblock that forms onion like micelles, which are accessible to cross-linking through disulfide bonds, also suitable for intracellural delivery [56].

The structure of the polymeric nanostructure has been proven to play a significant role to the drug-carrier efficacy of a nanosystem. A comparative study has been presented by Tan et al. In this study, the authors compared the encapsulation and release of paclitaxel by two polymeric nanostructures of different architecture, namely AB_2 miktoarm star copolymers, of the types poly(ethylene glycol)-[poly(L-lactide)]$_2$ (PEG-(PLLA)$_2$) and poly(ethylene glycol)-[poly(D-lactide)]$_2$ (PEG-(PDLA)$_2$), which form hollow core spheres and nanofibers through stereocomplexation between poly(ethylene glycol)-b-poly(D-lactide) (PEG-b-PDLA) and poly(ethylene glycol)-b-poly(L-lactide) (PEG-b-PLLA) [57]. The observed drug loading was 12 and 40 wt%, respectively, showing the effect of nanostructure morphology on the loading capacity of a hydrophobic drug. Interestingly, the drug release profile was similar for the two nanostructures, without an initial rapid increase of the amount of released drug.

Even though, drug delivery is the most well-studied research area in the frame of polymeric nanostructures with appealing bioapplications, polymeric self-assemblies have also been used as vehicles for the transportation of many other molecules with biological interest, like DNA, RNA, and proteins. Following this, Varkouhi et al. has proposed the use of a diblock, consisting of the stabilizing and stealthing PEO block and a high charge density cationic polyelectrolyte as potential carrier of siRNA and plasmid DNA. Due to the high charge density of the polyelectrolyte block, the DNA complexes are particularly stable, in terms of dissociation and transfection activity. Moreover, siRNA complexes were found to show low cytotoxicity, improved siRNA delivery and high gene silencing activity [58], illustrating the importance of the molecular characteristics of the polymer, in this case the high charge density, for the particular bioapplication. It has to be noted that the results were evaluated under the prism of the comparison among the block copolymer, a homopolymer of high charge density and a reference cationic polymer, namely PDMAEMA, Fig. 2.18.

Triblock terpolymers have also been used for gene delivery. As before, the complexation takes place through a cationic block (in this case PDMAEMA), while stability is ensured by a poly(ethylene glycol) methyl ether methacrylate block, while the third block is a pH-responsive copolymer of PDMAEMA and PBMA.

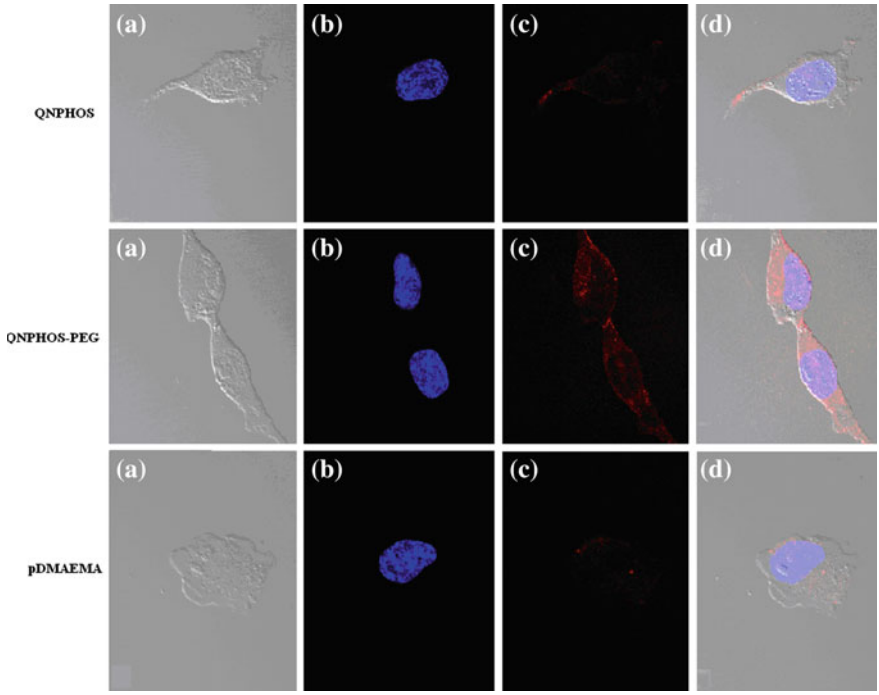

Fig. 2.18 Set of images which illustrate the successful gene transfer of RNA by cationic polymers. Confocal laser scanning microscopy images of cells incubated with complexes prepared with siRNA (*red*) and the homopolymer (QNPHOS), block copolymer (QNPHOS-PEO) or the reference polymer (PDMAEMA). **Images a** Pattern of cells with light microscopy. **Images b** stained Nuclei of cells. **Images c** Complexes with siRNA. **Images d** *Red* complexes with siRNA; *Blue* stained nuclei. Reproduced from [58] with permission from Elsevier

The above polymer forms particles of 86–216 nm upon complexation with mRNA. Depending of the relative position of each block, the polymer can be potentially used as an effective carrier due to the high transfection efficiency [59].

Besides the structure of the copolymer, its molecular characteristics seem to play a crucial role on the DNA effective transportation. Osada et al. have described this parameter very well by studying the effect of polylysine segment length, of a PEG-polylysine block copolymer, on the complexation with DNA. The obtained results indicate that packaging of plasmid DNA within both rod- and sphere-shaped polyplex micelles can be achieved. Interestingly, it was obvious that regularly folded plasmid DNA polyplex micelles are much more suitable for gene delivery than collapsed plasmid DNA polyplex micelles. The above indicates that controlling packaging of DNA, through control of the copolymer molecular characteristics, is crucial for achieving effective gene transfer [60].

Beyond the option of carrying a drug or a biomolecule in the body, the site-specific release is also an important parameter towards the formulation of an appealing nanosystem, as it was discussed above for the intracellular release of

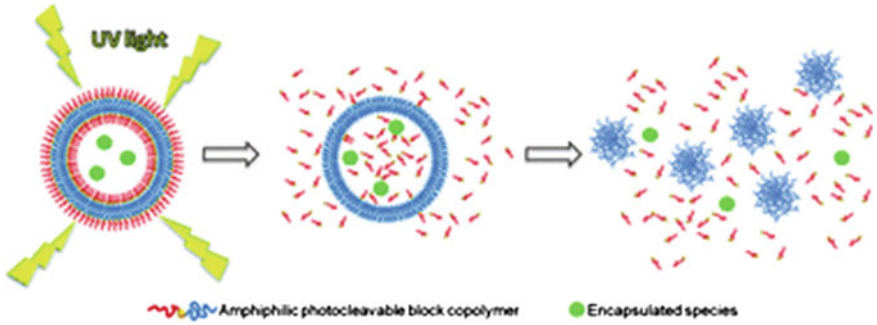

Fig. 2.19 Illustrative representation of encapsulated species release by a light-stimuli-responsive polymeric vesicle. Reproduced from [62] with permission from Royal Society of Chemistry

drugs. Pang et al. [61] have described the site-specific delivery of peptides to the brain using biodegradable polymersomes. Thiols, present in the monoclonal antibody OX26, were added to PEG-PCL polymersomes with maleimide functions on their surface. The used antibody was able to initiate endogenous receptor-mediated transcytosis of the polymersomes across the blood brain barrier, offering site-specific functionality.

Finally, the responsiveness of macromolecular self-assemblies to external stimuli is a very desirable property that increases the potential of block copolymer nanostructures for drug delivery. Toward this end, Cabane et al. [62] have presented the formation of polymeric vesicles as a light-triggered delivery system. In particular, they used a photocleavable amphiphilic block copolymer composed of PAA and poly(methyl caprolactone) as hydrophilic and hydrophobic blocks, respectively, linked by O-nitrobenzyl photocleavable segment for the formation of the vesicles. The above nanostructure is disintegrated upon UV irradiation and is rearranged to spherical aggregates, by simultaneous release of the encapsulated loaded molecules, Fig. 2.19.

Conclusions and Outlook

The amphiphilic copolymers in selective solvents self-assemble into nano-sized structures due to differences in the physicochemical characteristics of the constituent moieties. Spherical and cylindrical core–corona micelles as well as vesicles or polymersomes are the most commonly observed morphologies. Other morphologies such as toroids, bicontinuous, and multicompartment micelles, tubules, disk-like micelles, etc., are considered somewhat exotic. These structures are less frequently observed, however, they are not worth less than the commonly observed ones. The constituent moieties can be made sensitive to variations of parameters of the surrounding media such as temperature, pH, ionic strength, presence of specific additives/substances,

pressure, etc., so that the resulting interactions, properties of the self-assembled structures, morphology and morphological transitions can become sufficiently complex, versatile, and tunable, which is very important from a technological point of view.

The co-assembly is an approach to influence and modify the properties of the nanoassemblies, which allows expanding the utility of the latter. Different types of forces like hydrophobic interactions, electrostatic interactions, hydrogen bonding, donor-acceptor interactions, metal-ligand coordination bonds, etc., have been found to facilitate the formation of mixed, hybrid structures and to contribute to the introduction of new functionality and properties. The incorporation of additional entities even in small amounts in the mixed/hybrid nanostructures is an excellent approach to tune the aggregate morphology and to significantly alter the aggregate characteristics [2].

The nano-sized polymer structures have proven biomedical applications. Coupled with novel strategies for targeting, biodegradability, stimuli-responsiveness, controlled release, these nanoassemblies exhibit tremendous potential for delivery of, e.g., anti-tumor agents, genetic material, proteins, and other biologically active substances. The required extracellular and intracellular delivery of therapeutic molecules into the disease-associated cells presents the primary roadblock for enhancement of therapeutic effects of these molecules. Therefore, the proper selection and design of specific copolymers or nanoassemblies as well as the design of adequate delivery technologies have utmost importance. The structures typically contain surface domains that shield against undesired biological interactions and enable specific host cell receptor binding as well as elements/moieties for controlled delivery functions. These multistep tasks constitute an attractive challenge for the polymer researchers.

The polymerization-induced self-assembly is a facile, efficient, and reproducible strategy for preparation of families of polymeric nanoparticles having various morphologies via a one-pot process. Direct preparation of the block copolymer aggregates in the dispersion and emulsion polymerizations at high concentration of solids provides the possibility for large-scale industrial production. Preferentially, the polymerization-induced self-assembly should be conducted in aqueous media at quantitative monomer conversion.

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