Cross-linked block copolymer micelles: functional nanostructures of great potential and versatility

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Supramolecular self assembly techniques have provided a versatile means by which to selectively assemble polymer molecules into well-defined three dimensional core–shell nanostructures. The covalent stabilisation and tailoring of these dynamic nanostructures can be achieved using a range of chemistries within the assembly to afford robust functional nanoparticles. Many examples of the stabilisation, functionalisation and decoration of these nanoparticles have been reported in the literature and this *tutorial review* will focus on these recent developments and highlight their potential applications.

Introduction

The desire to control the composition, structure, and function of organic materials on the nanometre size scale has received increasing interest due to their wide-ranging applications from medicine to microelectronics. To this end, control of macromolecular size and shape has been achieved using both covalent and non-covalent interactions. The application of these interactions to form structures with large nanoscale dimensions requires the assembly of numerous small molecule

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Craig J. Hawker, PhD is currently Director of the Materials Research Laboratory and a Professor of Chemistry, Biochemistry and Materials at the University of California, Santa Barbara. From 1993–2004 he was a Research Staff Member and an investigator in the NSF Center for Polymer Interfaces and Macromolecular Assemblies at the IBM Almaden Research Center. He received a BSc (Hons) degree and University Medal in chemistry from the University of Queensland in 1984 and a PhD in bioorganic chemistry from the University of Cambridge in 1988 under the supervision of Prof. Sir Alan Battersby. Jumping into the world of polymer chemistry, he undertook a post-doctoral fellowship with Prof. Jean Fréchet at Cornell University from 1988 to 1990 and then returned to the University of Queensland as a Queen Elizabeth II Fellow from 1991 to 1993. He has been honored by numerous awards including the 2005 ACS Award in Applied Polymer Science from the American Chemical Society and the 2005 Dutch Polymer Award. Prof. Hawker is Editor of the Journal of Polymer Science, Part A: Polymer Chemistry. His research has focused on the interface between organic and polymer chemistry with emphasis on the design, synthesis, and application of well-defined macromolecular structures in biotechnology, microelectronics and surface science.

precursors. To facilitate the synthesis of these nanostructures as well-defined entities, methodologies have been developed to utilise covalent interactions to form robust building blocks, such as block copolymers. These can be designed to undergo a second assembly step *via* non-covalent interactions, with a relatively small number of components, to form nanostructures, such as polymer micelles. This general process has been called the dimensional evolution of synthetic organic chemistry.[1]

Aided by the recent advances in precision polymer synthesis, *via* developments in controlled radical polymerisation (CRP) techniques, there is great opportunity in terms of flexibility, diversity and functionality in the design of the polymeric building blocks. This versatility allows for control over their self assembly into nanoscale objects of varying size and shape. In particular, the application of these techniques towards the facile synthesis of well-defined functionalised hydrophobic and hydrophilic (co)polymers represents an important advance in the field of polymer science, enabling the construction of polymers with a high degree of control over molecular weight, sequence and end functionalisation, for utilisation in the self assembly of well-defined nanostructures. The desire to develop well-defined synthetic macromolecules is driven by the target of achieving a high level of control over the self assembly process, leading to materials with functional group complexity and structural precision similar to natural systems.

Karen L. Wooley

Karen L. Wooley received a Bachelors of Science degree in Chemistry from Oregon State University in 1988 and then studied under the direction of Professor Jean M. J. Fréchet at Cornell University, obtaining a PhD in polymer/organic chemistry in 1993. She then began an academic career as an Assistant Professor of Chemistry at Washington University in St. Louis, Missouri, and was promoted in 1999 to Full Professor with tenure. In 2006, Karen was installed as a James S. McDonnell Distinguished University Professor in Arts & Sciences. She has received young investigator awards from the National Science Foundation (1994–99), the Army Research Office (1996–99), and the Office of Naval Research (1998–01). Karen was named as a DuPont Young Professor (1996–99) and she received a 2002 Arthur C. Cope Young Scholar Award. In 2002, she also was awarded an NSF Division of Materials Research, Special Creativity Extension and she was the recipient of the Academy of Science of Saint Louis Innovation Award. In 2005, she received a Washington University Distinguished Faculty Award. Karen currently serves as an Editor for the Journal of Polymer Science, Part A: Polymer Chemistry, and as a member of the Editorial Advisory boards of Langmuir, Nano Letters, International Journal for Nanomedicine, Soft Matter and Supramolecular Chemistry. Research interests include the synthesis and characterisation of degradable polymers, unique macromolecular architectures and complex polymer assemblies.

Many nanostructure architectures such as spheres, toroids, helices, rods, disks, vesicles and tubes can be accessed by controlling the conditions under which diblock copolymer solution-state self assembly is conducted. The ability to control the assembly provides an exciting opportunity in the manufacture of unique materials that demonstrate properties that are not otherwise accessible by conventional techniques. In this review, we constrain ourselves primarily to the assembly, stabilisation and functionalisation of amphiphilic diblock copolymers into spherical nanostructures.

Spherical polymer micelles and nanoparticles are perhaps the most accessible assemblies, leading to significant potential in applications ranging from delivery vehicles for therapeutics, molecular imaging agents and as precursors to nano-sized microelectronic devices.[2] Often this diverse spectrum of applications requires both robust, stabilised structures and also the covalent attachment of functional molecules to the nanoparticle scaffold. The ability to tailor the physical, chemical and biological properties of these various nanostructures is central in the development of their potential applications. Thus, there has been great recent interest in the development of methodologies for the synthesis, cross-linking and functionalisation of polymer micelles. These functional and stabilised synthetic nanostructures can be compared to natural viral architectures, as both moieties possess self-assembled shells, have similar length scales and are capable of cell selective targeting. Given this analogy, the design of complex synthetic nanoparticles uses techniques that are based primarily on the basic construction tools found in nature—a combination and balance of weak and strong interactions, such as hydrophobic effects, electrostatic interactions, and covalent bonds.

There are numerous other classes of core–shell structures, such as monolayer/polymer brush protected and inorganic nanoparticles or quantum dots, which are of interest for a number of biological and medical applications. However, these inorganic hybrid nanoparticles will not be covered in this article and instead readers are directed towards recent reviews in these areas.[3–5] In addition, extensive and pioneering work has been performed in recent years by Armes[6] in the development of zwitterionic and totally hydrophilic stabilised polymer micelles, but as these constitute a whole category of nanostructures in themselves, they will not be included in extensive detail in this review. The development of these schizophrenic micelles is of particular interest given the ability to tune these materials by changing external solution conditions such as pH, temperature or electrolyte concentration. This in principle allows for better control over the encapsulation and delivery behaviour of these polymer micelles in aqueous media.

Solution self assembly of block copolymer micelles

The simplest and most common method for the assembly of synthetic polymer chains into well-defined nano- and micrometer dimensions involves the solution formation of polymer micelles (Fig. 1).[7] Polymer micelles are formed spontaneously *via* the solution-state self assembly of amphiphilic multi-block copolymers, which consist of hydrophobic and hydrophilic chain segments. This process involves the association of an

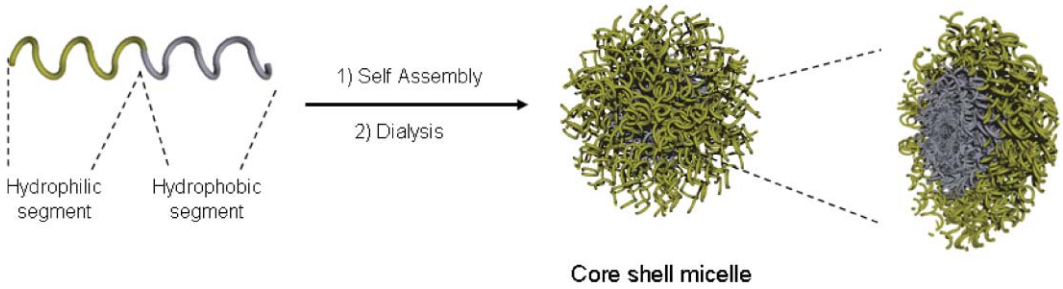

Fig. 1 Solution-state self assembly of amphiphilic diblock copolymers into spherical micelles.

insoluble segment or segment(s) of the copolymer chain, when the copolymer is placed in a solvent system that solvates only a portion of the overall chain, at concentrations at or above the critical micelle concentration (cmc). Experimentally, micelle formation is achieved by dissolving the amphiphilic copolymer in a good solvent for all of the blocks and then gradually adding a non-solvent for one of the blocks, followed by complete replacement of the good solvent by the non-solvent *via* dialysis. This process can produce well-defined aggregates, known as polymer micelles, which in aqueous solution consist of a hydrophobic core domain surrounded by a hydrophilic corona or shell layer. Conversely, inverse structures are also accessible and are known as reverse micelles.[8] These can be formed by adding a non-solvent for the hydrophilic block to afford the opposite of a conventional micelle, for which the hydrophilic core is surrounded by a hydrophobic shell in a hydrophobic surrounding media. However, only aqueous systems will be considered in this review.

The major driving force for the self assembly of amphiphilic copolymers is the decrease in free energy of the system due to the removal of the hydrophobic fragments from the incompatible aqueous environment by the formation of a micelle core stabilised and 'protected' from the surrounding aqueous media by the hydrophilic blocks—often called the hydrophobic effect.

The nature of the self assembly process allows for significant versatility in the chemical nature of the polymer micelles and thus permits fine tuning of the material properties, shapes and sizes. For example, the core composition can be varied to include glassy, crystalline or fluid-like materials, hydrolysed to produce an entirely hydrophilic structure, or fully degraded and removed to afford a solvent filled nanocage. Additionally, the shell layer can be positively- or negatively-charged or neutral, and contain reactive functional groups following the cross-linking reaction throughout the shell domain, to allow for the subsequent stabilisation of the micelle. The ability to control particle diameters readily in the range of 10–100 nm by adjusting the block copolymer chain lengths and the ability to establish a hollow polymer nanocage (of similar dimensions as biologically functional transport nanomaterials, *e.g.* virus particles) by removal of the core domain represents a significant advantage over other constructs, such as linear polymers, surfactants or dendrimers. In addition, polymer micelles have been demonstrated to be generally more stable and also often have a significantly lower cmc than small molecule surfactant micelles as well as offering more flexibility and diversity in their synthesis. Another class of polymer micelles that will not be discussed in this review, but represents an important development in the field of supramolecular self assembly, is that of polyion complex (PIC) micelles, which were first developed by Kataoka.[9] These PIC micelles were prepared through electrostatic interactions between a pair of oppositely charged block copolymers in which both contained a neutral poly(ethylene) glycol segment. The authors have utilised these PIC micelles as vehicles for the delivery of charged compounds and for the sequestration of charged moieties such as proteins and peptides.

Various nanostructures can be formed by tailoring the block lengths, compositions and self assembly conditions; however, this review will focus solely on the formation of spherical micelles. These spherical micelles possess a unique core–shell morphology, which can be modified both chemically and structurally by the incorporation of cross-linking and/or functional groups at selective locations within the nanostructure. The characterisation of the size, shape and distribution of these polymer micelles can be achieved using a plethora of techniques such as dynamic light scattering (DLS), transmission electron microscopy (TEM), atomic force microscopy (AFM), analytical ultracentrifugation, viscometry, size exclusion chromatography (SEC) and small angle neutron scattering (SANS).

Stabilisation by cross-linking

A major disadvantage of micelles is their dynamic nature which leads to instabilities at high temperature, at low concentrations and under certain changes in solvent conditions. As a result, there has been significant interest in the stabilisation of micelles and in particular, polymer micelles. In nature, stabilisation of self-assembled structures is often achieved by covalent cross-links and, by analogy, it has been demonstrated that the selective formation of such cross-links between specific blocks within a copolymer micelle is possible and affords a single, nanostructured macromolecule or nanoparticle. Thus, the formation of cross-links throughout a specific domain offers stability to the nanostructured assemblies by providing reinforcement to the weak intermolecular interactions that facilitate polymer micelle assembly and existence. There are several potential locations for cross-linking within diblock polymer micelles, including at the core chain end, within the core domain, at the core–shell interface, throughout the shell layer, and on the surface (Fig. 2). The location of this cross-linked domain can dramatically affect the physical and chemical properties of the resulting materials. In

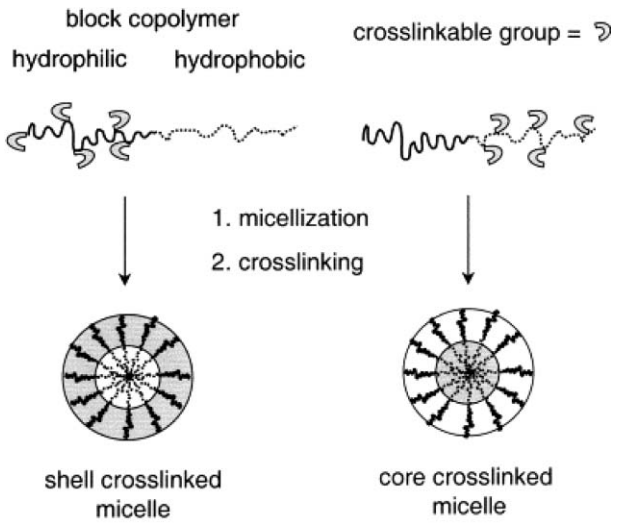

Fig. 2 Schematic representation of the different types of functional amphiphilic block copolymers utilised in the formation of shell cross-linked or core cross-linked nanoparticles. (Reproduced with permission from Ref. 10. Copyright 2001 Elsevier.)

addition to the method employed, the extent of the cross-linking can also impact the stability, structure and thus applications of the resultant materials.

The primary chemistries employed for core cross-linking and stabilisation of the micellar particles are: the incorporation of polymerisable or photo/UV cross-linkable groups; the introduction of cross-linking reagents; and the introduction of external stimuli. In 1979 the groups of Prochaska and Baloch were the first to introduce the concept of stabilising a polymer micellar assembly by cross-linking throughout the core, with this case consisting of polybutadiene, using photochemical irradiation.[11] Following this ground breaking report, cross-linking chemistry has been used extensively to stabilise micellar constructs.[12] For example, in 1999 a methacryloyl chain end functionalised poly(ethylene) glycol-*b*-polylactide could be polymerised selectively to afford a stabilised micelle structure.[13] As an alternative cross-linking strategy, many groups have reported the utilisation of difunctional cross-linking reagents, which allow for the selective covalent stabilisation of the core domain.[14] Recently, responsive core cross-linking has been reported by the application of external stimuli such as pH, temperature or the addition of metal salts (Fig. 3).[15–17]

Chemical fixation of either the core or shell is of interest, due to the increased stability of the nanoparticles relative to their precursor micelles. It is has also been proposed[10] and demonstrated[18] that cross-linking the core or shell domain alters the permeability of the respective domain and thus significantly affects their potential as delivery vehicles. Significant work in this area has focused on the cross-linking of the shell domain through functionalities located directly on the coronal polymer backbone, thus affording a central core domain comprised of linear chains that are covalently linked to the inner surface of the permeable cross-linked shell layer. These shell cross-linked nanostructures are envisaged to have greater application than their core stabilised counterparts, due to their higher core mobility, greater versatility in the core composition and properties, and membrane-like characteristics of the cross-linked shell layer, which improves their encapsulation and surface binding potential. The cross-linking of the shell was first reported using radical polymerisation techniques[19] and then by condensation reactions,[20] both of which generate robust nanostructures which contain a permeable corona layer. These methodologies allow for the complete coverage of the core domain by the uniform stabilised membrane shell layer, whilst maintaining the desired nanoscale particle diameters with narrow size distributions. However, the application of this chemistry can be limited by the required preparation of an appropriate amphiphilic diblock copolymer. Despite this, the versatility in the nanostructure synthesis allows for the tuning of the structure and chemical composition of the nanoparticles by using the wide range of synthetically available block copolymers. The shell cross-linking of micelles is commonly achieved using a diamine

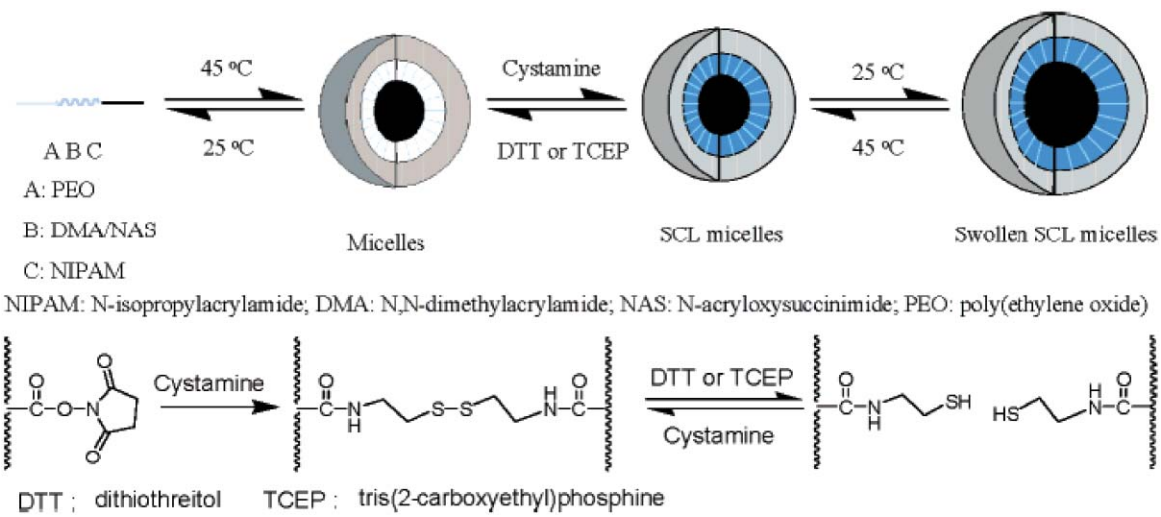

Fig. 3 Schematic illustration of the formation of reversible shell cross-linked micelles from PEO-*b*-(DMA-*s*-NAS)-*b*-NIPAM triblock copolymers. (Reproduced with permission from Ref. 16. Copyright 2006 American Chemical Society.)

cross-linker which can react in aqueous media in the presence of a carbodiimide activator with the carboxylic acid groups of the poly(acrylic) acid polymer block which comprises the shell layer. Alternatively, alkylation chemistry has been used for the cross-linking of the shell layer consisting of hydrophilic monomers such as *N*,*N*-dimethylaminoethyl methacrylate.[21] A broad range of polymers have been utilised as the core hydrophobic domain of shell cross-linked nanoparticles and include styrene, isoprene, butadiene, caprolactone and methyl acrylate, amongst many others. Monomers that have been used to prepare the hydrophilic water soluble domain include, amongst others, 4-vinylpyridine, (meth)acrylic acid and 2-dimethylaminoethyl methacrylate.

It is important that the cross-links are limited to intramicellar reactions rather than intermicellar couplings, and this limitation is achieved by performing the cross-linking at high diblock copolymer dilution or by performing the cross-linking reactions within an intermediate layer with steric stabilisation provided by polymer chain segments extending to the nanostructure surfaces,[22] to avoid the formation of large covalently bound aggregates. These shell cross-linked nanoparticles possess a degree of structural organisation—having a well defined external shell, an interfacial layer and a central core consisting of linear polymer chains—thus these nanoparticles consist of three distinct nano-domains, in which further chemistry can be performed. The potential uses of these cross-linked materials are significantly improved by their increased stability towards environmental changes such as concentration and temperature, compared to their non-cross-linked micellar precursors. The additional subsequent covalent attachment of moieties to the cross-linked self-assembled architectures further extends the potential application of these materials. For example, if such covalently stabilised nanoparticles are manipulated chemically, their drug delivery potential can be improved by the attachment of specific ligands to their surface or within their core domain. An example of the secondary modification of these shell cross-linked nanoparticles, which has been demonstrated in the literature, involves the cleavage of the core polymer chains, followed by dissolution of the linear core chains and excavation of the nanoparticle core to afford a cross-linked polymer shell or nanocage.[23] The application and manipulation of these nanocages are beyond the scope of this review, but it is significant to note that they constitute another important genre of nanostructured materials which are derived from shell cross-linked nanoparticles.

Armes has elegantly utilised the concepts of core and shell cross-linking to create hybrid type nanoparticles, in which the central layer of a triblock copolymer is cross-linked. These three layer onion cross-linked micelles are obtained from a triblock copolymer, in which the central block (*e.g.* (*N*,*N*-dimethylamino)ethyl methacrylate) has reactive groups capable of undergoing cross-linking to afford a central core of linear chains surrounded by a cross-linked layer, that is further surrounded by a corona of linear polymer chains (Fig. 4).[22] These triblock copolymers offer the advantage over conventional diblock copolymers that they allow inner-shell cross-linking to be carried out at high polymer concentrations without intermicellar cross-linking and aggregation. This quality, in addition to the tunable nature of the micelles

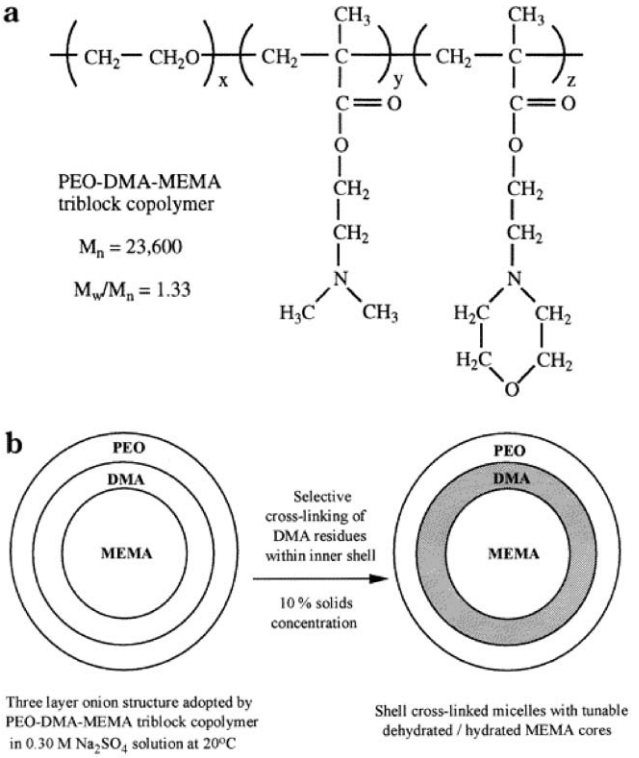

Fig. 4 Chemical structure and schematic illustration for the formation of shell cross-linked micelles formed at high solid concentrations using PEO-DMA-MEMA triblock copolymers. (Reproduced with permission from Ref. 22. Copyright 2000 American Chemical Society.)

structures, has been proposed to facilitate laboratory scale up and improve commercial viability of these polymer micelles.

The stabilisation of polymeric micelles *via* cross-linking reactions is an important aspect of the application of these materials. However, cross-linking chemistries are inherently inefficient and hard to quantify experimentally. This is especially the case when the method of cross-linking is by the introduction of a difunctional reagent which must react with functionality on the same chain. Thus the development of new cross-linking strategies that circumvent these problems is an important objective in the application and development of stabilised polymer micelles.

An important development in the field of polymer micelle stabilisation was the development of reversible cross-linking chemistries, which are beneficial in the applications of these materials as drug delivery vehicles. The reversible cross-linking of a poly(ethylene)glycol-*b*-poly(lysine) micelle was elegantly demonstrated by the group of Kataoka, by the selective introduction of thiol groups to a fraction of the lysine repeat units and then assembly to form a PIC micelle with a poly(α,β-aspartic acid) polymer. Upon assembly into spherical structures the thiol residues in the lysine core domain were oxidised to form disulfide linkages between polymer chains and afford a stabilised and robust core cross-linked micelle. These cross-links throughout the core domain are reversible as they contain disulfide bonds, which can be readily cleaved upon addition of a reducing agent such as dithiothreitol or glutathione. A similar approach was also recently reported by McCormick for the synthesis of reversibly shell cross-linked

micelles by amidation of an *N*-acryloxysuccinimide functionalised polymer with cystamine, which acts as a disulfide bifunctional primary amine.[16] The resultant amide linkage between cystamine and polymer is stable but the cystamine disulfide bond can be cleaved readily to impart reversible cross-linking characteristics to the nanoparticle (Fig. 3). These reversibly cross-linkable micelles have great potential in the field of drug delivery due to the selective cleavage of the disulfide bond, which can occur under reducing conditions within the target cell, thus enabling a controlled release and targeted delivery approach.

Subsequent chemical modification of cross-linked micelles

In analogy with the stabilisation of polymeric micelles, the introduction of functionality at various different locations within polymer micelles can be achieved, thereby allowing for the tailoring of these materials towards specific applications such as drug delivery vehicles. For a two-layered, core–shell nanoparticle, there are three major classes of functional domains (Fig. 5), and the number of unique functionalisation sites increases for each additional layer added to the complex nanoparticle structure. Moreover, within a core–shell structure, there can be additional phase-segregated domains within the core or the shell, for example the "bumpy" nanoparticles and multi-compartment micelles assembled from complex mixtures of block copolymers and homopolymers.[24] In the simplest, diblock copolymer core–shell morphology, the first class of nanoparticles contain functional groups at the surface of the particle (*via* either surface or shell functionalisation), which are envisaged to allow for the targeted and directed delivery of the vehicle to a particular site. In the second class, the substituents are located at the core–shell interface and are available as reactive handles for cross-linking of the corona or for the introduction of further functionality. The third class of functional nanoparticles contain reactive groups located in the hydrophobic core domain or at the hydrophobic polymer chain end and may be used to cross-link the core or introduce further functionality into the nanoparticle core. By judicious choice of the functionality introduced into the nanoparticles, these groups can be utilised as selective reactive handles and can be made to work cooperatively (*e.g.* acting as energy transfer-based detectors or coupled triggered release systems) to allow for the further tailoring of these materials towards specific applications.

One area of particular interest is that of bioconjugation or attachment of biological molecules to the polymer assemblies. This combination of natural and synthetic components to form a hybrid entity is the fundamental principle of bioconjugation. Bioconjugation is employed as an essential technique in the production of diagnostic and therapeutic products. The functionalisation of polymer materials with saccharides, peptides, oligonucleotides, targeting ligands, antibodies and other moieties has received considerable interest as a means to generate structures capable of polyvalent, specific binding interactions. As a result, the introduction of these functionalities into the polymer nanoscale assemblies is of increasing interest and application.

Surface (and shell) functionalisation *via* chain terminus functionality

Many of the advances in the surface functionalisation of polymer micelles and nanoparticles have focused on the incorporation of biomolecules such as saccharides and peptides, or reactive chemical handles. It is proposed that the attachment of various specific ligands to the hydrophilic chain terminus available on the nanoparticle surface could, in particular, be used to improve the targeting of micelles in drug delivery.

To date, two primary synthetic strategies for the introduction of surface accessible functional groups have been

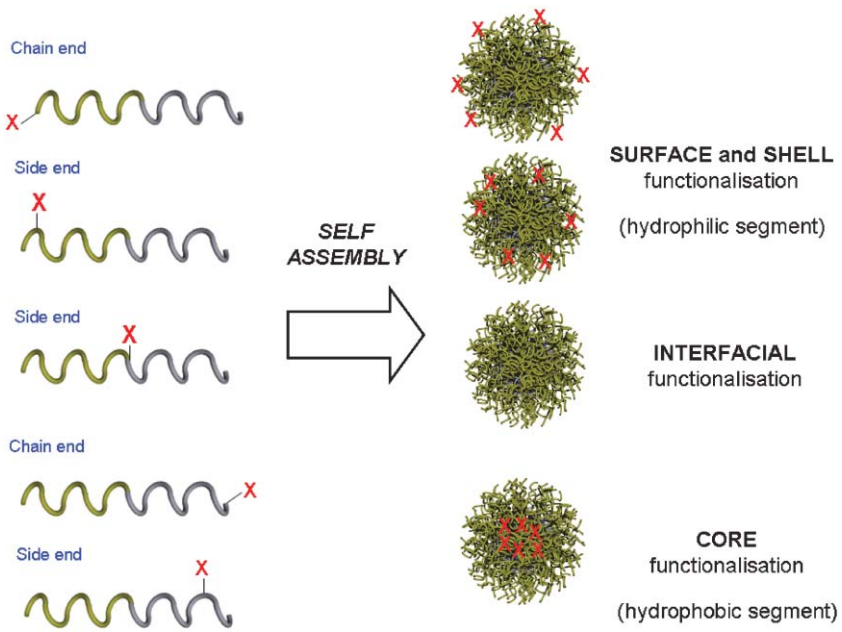

Fig. 5 Schematic to illustrate the possible locations of functionalisation within a spherical diblock polymer micelle.

established and involve either use of a functionalised initiator species or random incorporation of functionalisation in the shell layer post-preparation. The latter will be covered in detail in the shell functionalisation section of this review. The former strategy was first employed in 1999 by Kataoka for the synthesis of sugar chain end functionalised poly(ethylene glycol)-*b*-poly(D,L lactide) block copolymers[25] for the development of drug delivery micellar systems. The synthesis of these functionalised polymers required protecting-group chemistry for the chain end functionality, which was removed post-polymerisation and pre-assembly. Upon solution self assembly, regioselectively chain end functionalised micelles with narrow size distributions were isolated. The authors demonstrated that these sugar groups were surface available using the binding of RCA-lectin, which is known to selectively recognise sugar residues. Later, in 2003, Haddleton reported a similar strategy for the synthesis of sugar-coated micelles using more versatile CRP techniques, that did not require elaborate protection strategies (Fig. 6).[26] In this paper commercially available starting materials were used to prepare the glucose and galactose functionalised ATRP initiators in a single high yielding step.

More recently, Armes reported the chain end functionalisation of amphiphilic block copolymers with folic acid functionality and their assembly into pH-responsive micellar constructs for gene delivery applications.[27] The folate receptor, which has a high binding affinity for folic acid, has been identified as a tumor marker that is expressed at elevated levels in many types of cancer cells, relative to normal tissue. Therefore, by designing folic acid functionalised nanoparticles, which also contain a therapeutic payload, these may be able to selectively bind and deliver the payload to the over expressed target cells. The authors suggest that these micelles will selectively target folic acid receptors on tumor cells and enter the target cells by endocytosis. Once inside the tumor cells, the relatively low local pH of around 5.0, will cause the responsive micelles to dissociate (*via* protonation of the hydrophobic block and conversion to a hydrophilic polymer) and cause release of the payload precisely where it is required. The development of responsive micellar and nanoparticle constructs represents an important advance in the application of these materials as selective delivery vehicles.

In 2004, Wooley reported using CRP techniques for the synthesis of chain end sugar functionalised diblock copolymers that could be self-assembled using a mixed micelle strategy and cross-linked throughout the shell layer to afford nanoparticles in which mannose functionality was located selectively throughout the particles' shells and presented from their surfaces.[28] These saccharide functionalised particles were designed as polyvalent nanoscaffolds for selective interactions with receptors on Gram negative bacteria. The surface availability and bioactivity of the mannose units was confirmed using agglutination inhibition assays and TEM studies with bacterial cells. This study was extended to include biotin and antigen functionalised nanoparticles, in which the available biomolecules at the surface of the nanoparticle were quantified using a competitive binding assay, fluorescence correlation spectroscopy and degranulation assays respectively (Fig. 7).[29,30]

The innovative mixed micelle strategy utilised in these experiments involves the co-micellisation of two amphiphilic block copolymers of similar composition, whereby one contains functional groups at the hydrophilic chain terminus and the other does not contain chain end functionality. It was found that the degree of surface coverage could be tailored by

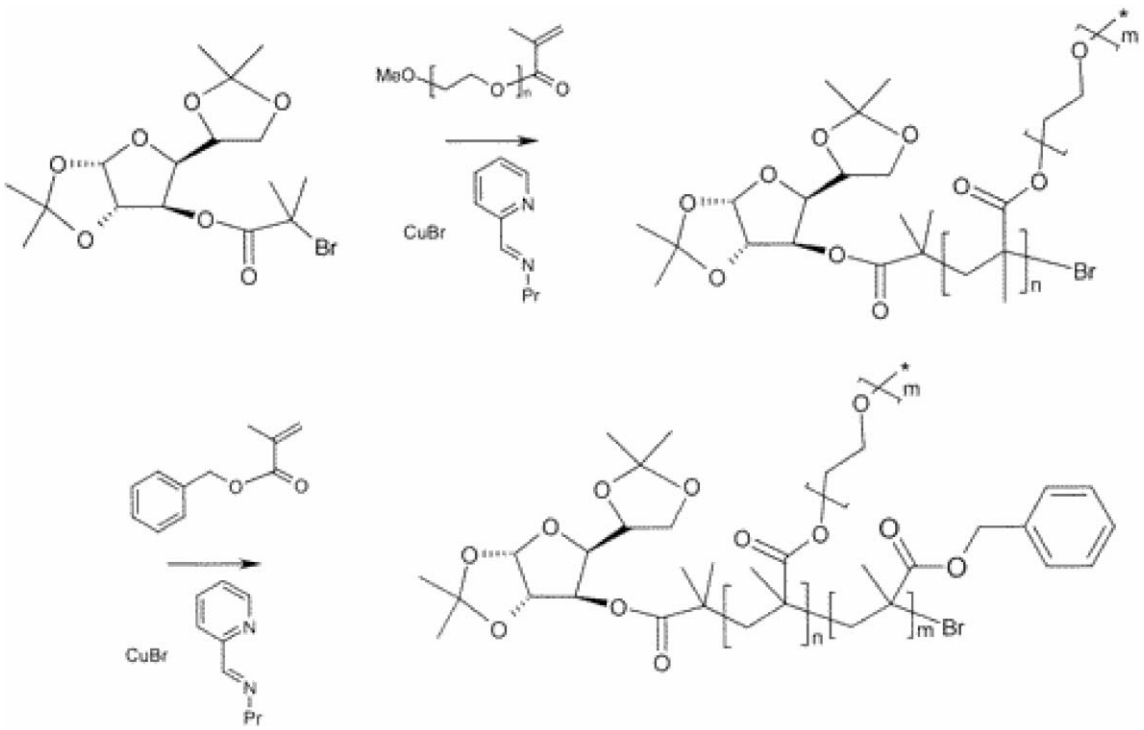

Fig. 6 Synthesis of glucose and galactose chain end functionalised amphiphilic polymers by ATRP. (Reproduced with permission from Ref. 26. Copyright 2003 American Chemical Society.)

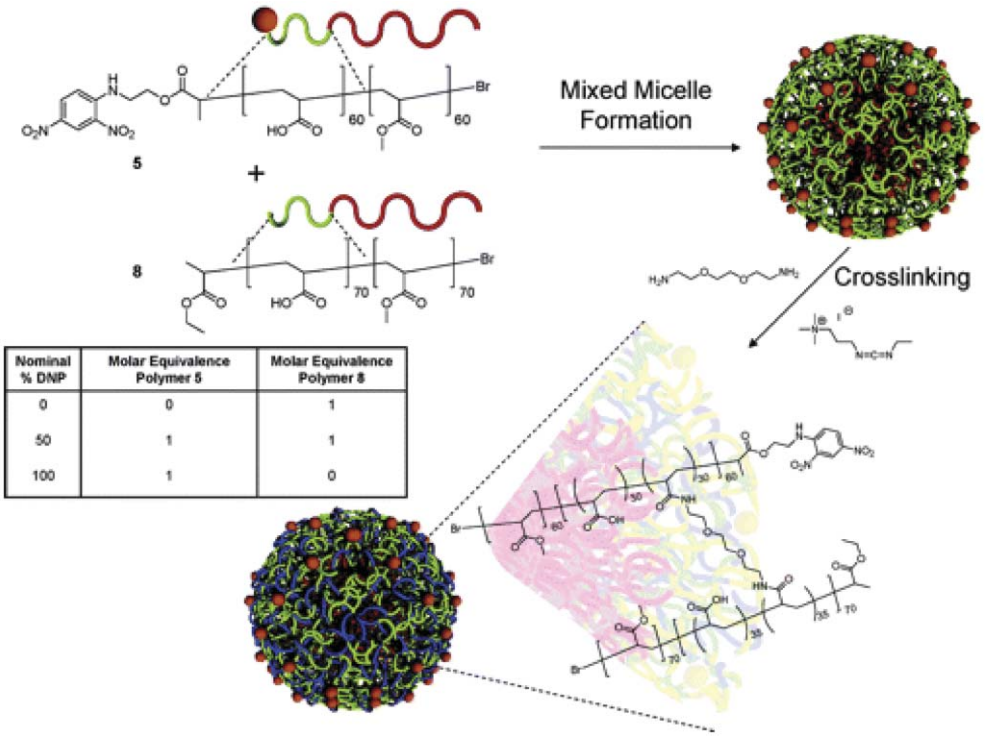

Fig. 7 Construction of 0%, 50% and 100% dinitrophenylated micelles and shell cross-linked nanoparticles using a mixed micelle strategy. (Reproduced with permission from Ref. 30. Copyright 2005 American Chemical Society.)

altering the stoichiometric ratio of the chain end functionalised and non-functionalised block copolymer precursors, using the quantitative studies reported in these papers. This approach allows for the incorporation and tuning of varying amounts of functional groups presented at the nanoparticle surface. However, the biotinylated nanoparticle study indicated that the degree of surface available functionality was only around 25% of the predicted value from theoretical studies, which was attributed to a loss of functional group availability in the micellisation and shell cross-linking processes. The inefficiency in end group incorporation in polymer micelles represents a significant obstacle in the future application of these materials and consequently new routes towards the quantitative introduction of functional groups must be developed.

An alternative strategy for the chain end functionalisation of micelles and nanoparticles was reported in 2003.[31] This involved the preparation of peptide bioconjugated amphiphilic polymers using a CRP initiator coupled to the peptide termini on Wang's resin, followed by the subsequent application of CRP techniques to grow block copolymers from the solid support. These chain end peptide functionalised synthetic block copolymers could then be cleaved from the resin and assembled into micelles. These peptide polymer bioconjugates were found to demonstrate enhanced anti-microbial activity relative to free peptide; this was the first indication that the peptide motif was both bioavailable and bioactive when conjugated to the polymer nanoparticle surface.[32]

Overall the chain end functionalised strategy has been demonstrated to afford nanoparticles with surface- and bio-available functional groups, however the degree of control associated with randomly incorporating functional groups into the hydrophilic shell of nanoparticles is low and the exact efficiency of presenting these molecular recognition elements on the nanoparticle surface (in competition with burying of the groups beneath the surface, due to various polymer chain segment conformations that can be adopted) is unknown. Similarly, a significant disadvantage of the functionalised initiator strategy is that complex synthetic routes are often required to obtain the desired functionality at the hydrophilic polymer chain end and the synthesis of a different initiator is required for each desired functional polymer nanoparticle. As a result this strategy is often limited by synthetic challenges and possible incompatibility with the polymerisation conditions, thus requiring complex protection strategies and further restricting the range of functional groups that can be incorporated.

To broaden the range of surface functional groups and to maximise their presentation at the nanoparticle surface, a versatile approach involving a common functionalised initiator leading to surface-available chemical handles designed for facile attachment of desired moieties to the surface functionalised micelle/nanoparticle was developed. Given that the application and reaction of these functionalised nanoparticles in the study of biological processes depends on two key factors, orthogonality and selectively in the relevant physiological settings, it was proposed that the non-reactive nature of azides and alkynes towards biological molecules should enable these functionalities to behave as inert chemical handles for diverse chemical functionalisation of the nanoparticles. As a result, a particularly attractive route towards the functionalisation of these water soluble materials is the copper(I)-catalysed Huisgen 1,3-dipolar cycloaddition between azides

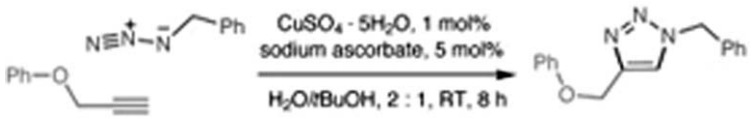

Fig. 8 Example of the regiospecific copper(I)-catalysed 'Click' reaction of a terminal alkynyl and an azido functionality. (Reproduced with permission from Ref. 33. Copyright 2001 Wiley-VCH.)

and alkynes to regioselectively yield 1,2,3-triazoles (Fig. 8). Attractive features of this reaction are that it is highly efficient, selective and proceeds under benign reaction conditions.[33] In addition, this cycloaddition reaction has shown excellent compatibility and orthogonality with a wide range of functional groups including bioactive molecules and, as a result, the application of this chemistry is of great current interest. Consequently, there has been significant recent effort in the functionalisation of biomolecules, surfaces, inorganic substrates, and polymers using 'Click' chemistry.[34]

To exploit the potential of 'Click' chemistry requires the synthesis of alkynyl or azido functionalised amphiphilic block copolymers from 'Click' functionalised CRP initiators. These functional block copolymers can then be assembled supramolecularly using a mixed micelle methodology and cross-linked, using conventional chemistries, to afford 'Clickable' chain end functionalised nanoparticles. These nanoparticles were recently demonstrated to be capable of being transformed under 'Click' conditions, using the complementary 'Click' reagent, into a range of surface functionalised micelles and nanoparticles. The orthogonality of 'Click' chemistry allowed functionalisation to be performed post self-assembly and cross-linking to ensure maximum presentation and fidelity of the chain end functional groups in the resultant nanoparticles (Fig. 9).[35] A significant synthetic advantage of this methodology is that protecting group chemistries for the introduction of the desired functional groups are not required, due to their introduction using 'Click' chemistry after both nanoparticle assembly and cross-linking. Initial experiments confirmed the quantitative incorporation of a hydrophilic fluorescent dye molecule. Meanwhile, further work is underway to confirm the availability of the functional groups at the nanoparticle surface compared to conventional mixed micellar strategies. Given the ever expanding field and interest in 'Click' chemistry, a wide range of biomolecules have been functionalised with azido and alkynyl groups and, thus, there exists great potential in the application of these chemistries for the surface functionalisation of polymer nanoparticles.

Shell (and surface) functionalisation *via* side chain functionalities

The shell layer can be considered as a swollen hydrogel extending from the core to the surface of the nanoparticle and reinforces the pre-assembled structure. The thickness and

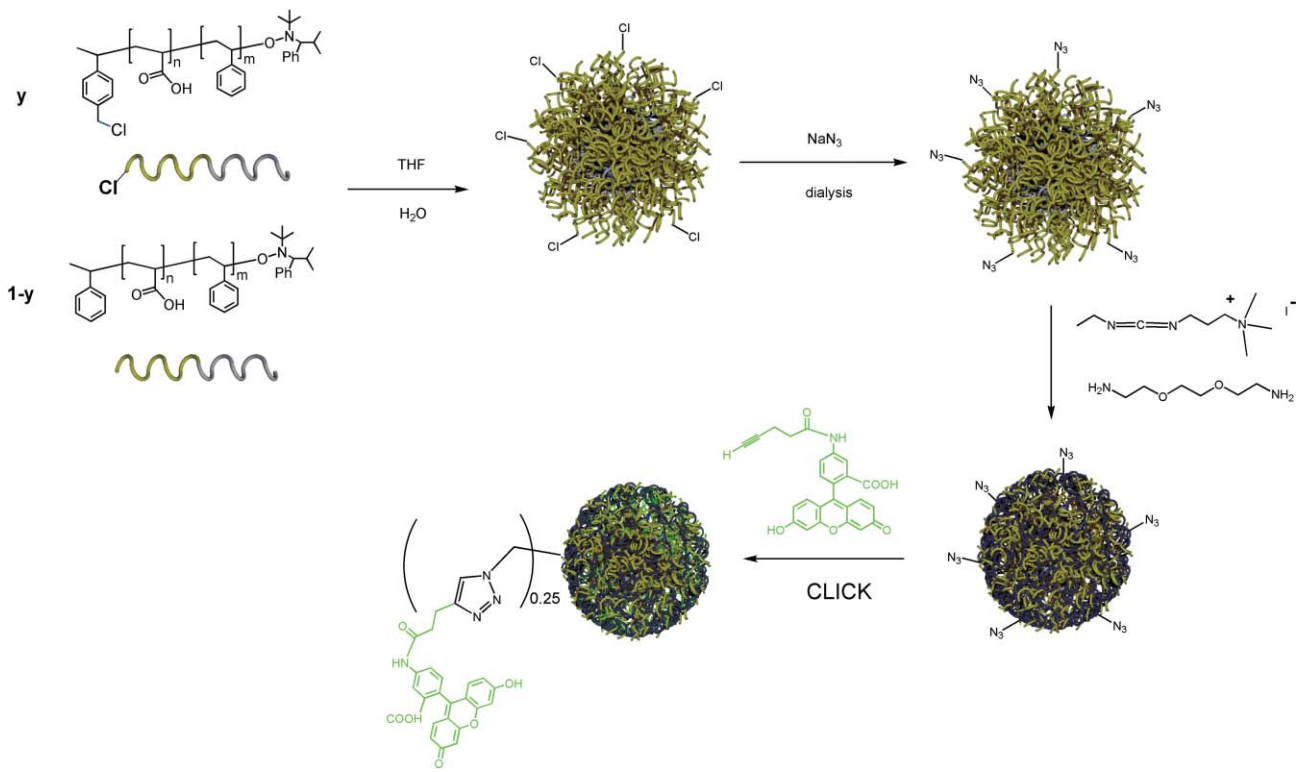

Fig. 9 Schematic illustrating the chain end functionalisation of block copolymer micelles using 'Click' functionalisation.

permeability of the shell layer controls both the access and release of guest molecules to and from the core domain. In addition, the shell composition determines the surface properties of the nanoparticle and this in turn affects the interaction of the nanoparticle with its surrounding environment and other substrates. The selective functionalisation of the shell domain is important as it has been demonstrated that by utilising a suitable tethering moiety, the functionality incorporated throughout the shell domain may be surface available. The primary strategy for the selective functionalisation of the shell domain involves chemical modification of the polymer backbone groups that comprise the shell layer on the hydrophilic block. This strategy has proven to be highly successful and has been employed extensively in micelles and nanoparticles containing reactive acrylic acid residues in the shell domain.

Initial studies focused on the shell modification of polymer nanoparticles and nanocages using solid support peptide chemistries (Fig. 10).[36] In this study the protein transduction domain (PTD) from the HIV-1 tat protein was prepared independently on a resin solid support. The amino chain end of the protein was then coupled to the acrylic acid residues in the nanoparticle shell domain. Following cleavage from the solid support and simultaneous core excavation by hydrolysis of the poly(caprolactone) segment, nanocage structures were obtained that contained a 'patch' of protein functionalisation upon the surface and/or within the shell layer. Fluorescent labelling using similar amidation chemistry throughout the shell layer afforded fluorescein tagged bioconjugate nanocages for investigation in cell binding and transduction.

Subsequently, numerous different functionalities such as folic acid[37] and $\alpha_v\beta_3$ targeting ligands[38] have been incorporated selectively into the shell domain by using straightforward and efficient amidation chemistry within the poly(acrylic) acid shell layer (Fig. 11). In the case of the folic acid nanoparticles the functional groups were conjugated with a short chain biocompatible polymer, poly(ethylene) glycol, to overcome steric issues and enable surface presentation of the interactive folate functionality. These folate-functionalised nanoparticles were found to undergo receptor-mediated endocytosis with folate-overexpressing KB cells *in vitro* and KB xenograft targeting *in vivo*.[39]

In most cases, the additional incorporation of a pH-dependent fluorescent dye molecule using similar chemistries was performed. This pH sensitive fluorophore can act as an optical marker to allow for tracking and characterisation of the nanoparticle when investigated *in vivo* and *in vitro*. All of these studies indicated that there was little or no change in hydrodynamic diameter of the nanoparticles upon functionalisation within the shell layer. For the peptide-functionalised nanoparticles, UV-vis spectroscopic and phenylglyoxal analyses were utilised to quantify the number of peptide molecules per particle. Following fluorescent labelling of the peptide functionalised nanoparticles, it was demonstrated that

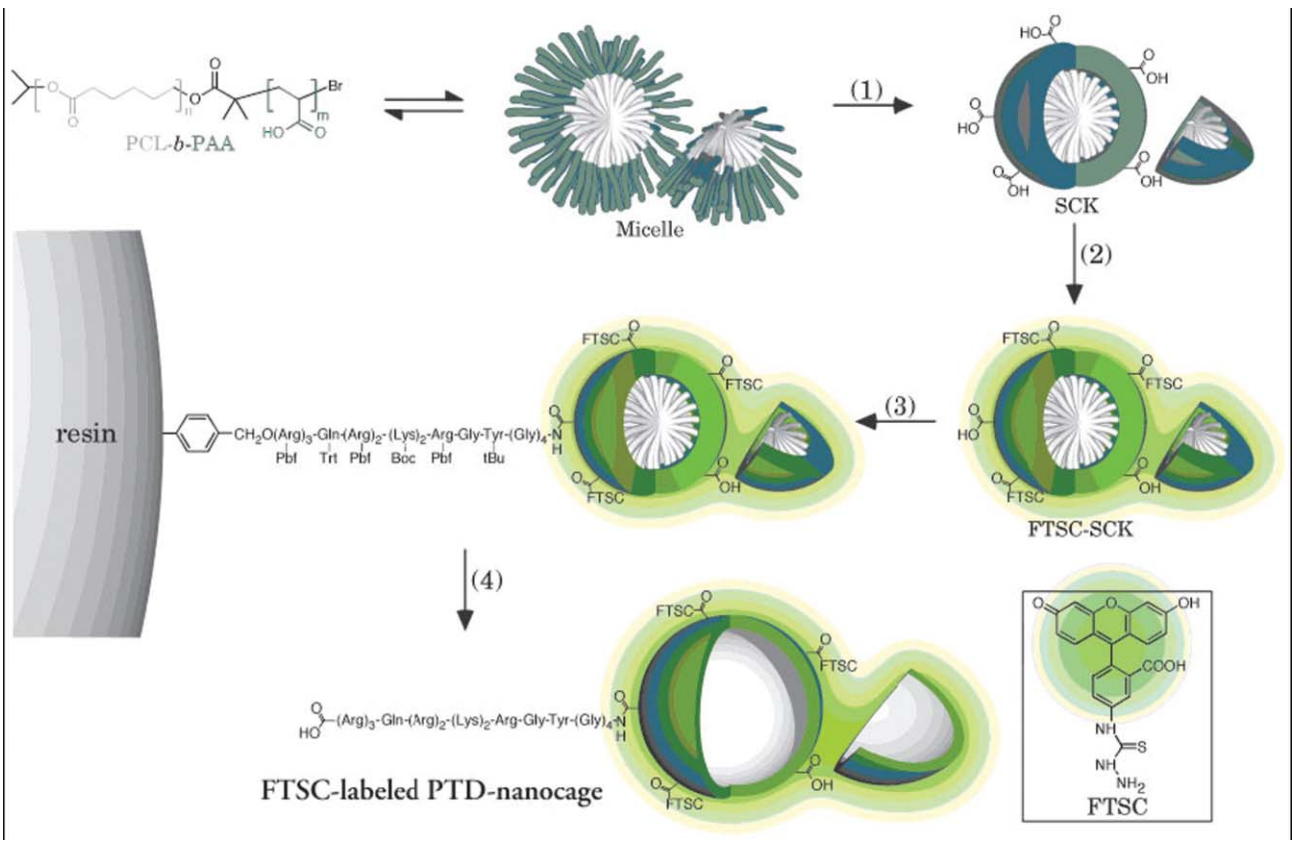

Fig. 10 Illustration of the synthetic strategy involving the coupling of a solid supported protein transduction domain (PTD) with a fluorescein labelled shell cross-linked nanoparticle, followed by cleavage from the support and core excavation. (Reproduced by permission from Ref. 36. Copyright 2001 American Chemical Society.)

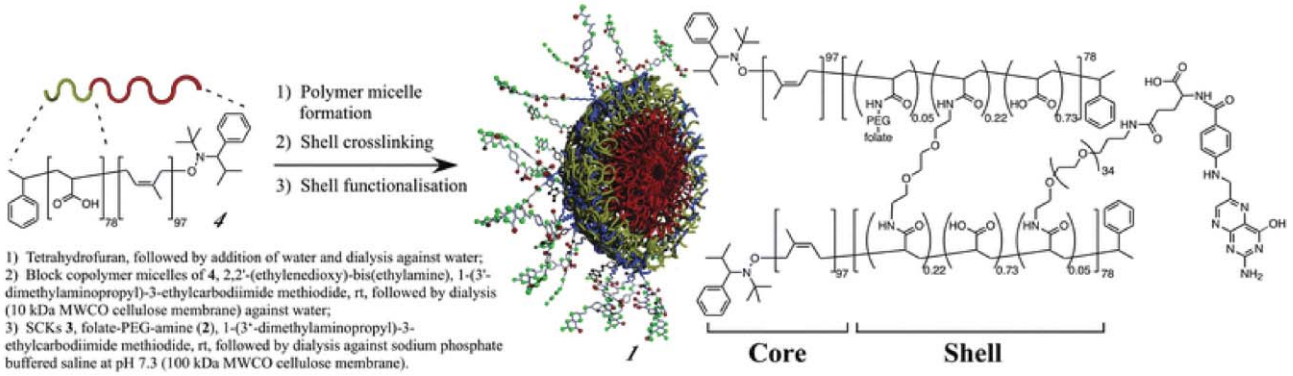

Fig. 11 Schematic representation of the folate functionalisation of a shell cross-linked nanoparticle, involving a three step methodology starting from the amphiphilic block copolymer. (Reproduced with permission from Ref. 37. Copyright 2003 American Chemical Society.)

the incorporation of the peptide residue into the shell layer facilitates the *in vitro* transduction of the nanoparticle across cellular membranes.

The concept of incorporating recognition units into the shell of cross-linked nanoparticles was further extended by controlled agglomeration, by the introduction of peptide nucleic acid (PNA) sequences onto the well-defined shell cross-linked nanoparticles by Wooley and Taylor.[40] These nanoparticles were synthesised by a stepwise assembly of poly(acrylic) acid-*b*-poly(styrene) copolymers into micelles, followed by covalent stabilisation to afford the nanoparticles. These nanoparticles were then PNA functionalised using amidation chemistry and finally assembled into higher order nanoparticle arrays by molecular recognition of the PNAs and using the PNA functionalised nanoparticles as building blocks. PNA sequences are known to undergo complementary binding to complementary DNA, RNA and PNA with high affinity and thus can be utilised to facilitate the selective supramolecular assembly of nanostructures of higher complexities. Using this non-covalent binding interaction of complementary PNA motifs, it was demonstrated that the mixing of nanoparticles labelled with complementary PNA sequences allowed for directed self assembly. This base pairing driven aggregation is both selective and tunable, which should allow for the design of specific hierarchical assemblies. The authors reported the selective and controlled fabrication of robust directed networks using stoichiometrically PNA functionalised nanoparticles.

Although these methods have proven successful and valuable, a unique and orthogonal strategy was recently developed that would allow the expansion of the types of chemistry that could be performed within the nanoparticle shell domain. In addition, this orthogonal approach does not require the creation of a unique functionalised initiator for each different functionality to be introduced into the nanoparticle. It is also designed to be tolerant of other functional groups present in the nanoparticle and to be unaffected by any further chemistry performed on the nanoparticle. In 2005, amidation reactions were performed within the hydrophilic poly(acrylic) acid chain segment of diblock copolymers selectively to incorporate 'Click' reactive azido and alkynyl handles (Fig. 12).[41] The introduction of the 'Click' groups could be performed before or after self assembly or cross-linking. The availability and reactivity of these functional groups towards 'Click' chemistry was demonstrated by reaction with complementary 'Click'-functionalised fluorescent dyes. This 'Click' functionalisation strategy was demonstrated to have the advantage of allowing simultaneous functionalisation and cross-linking throughout the shell layer with no loss in nanoparticle fidelity or uniformity. Analysis by analytical ultracentrifugation sedimentation equilibrium equipped with UV-vis detection optics confirmed the covalent attachment of the fluorescent molecules within the nanoparticle. In addition, analysis of the amount of dye successfully 'Clicked' into the nanoparticle shell layer was performed using first derivative UV-vis spectroscopic analysis and confirmed the quantitative nature of the 'Click' reaction within the nanoparticle shell.

Until recently, the introduction of functionality into the shell domain within acidic coronas was limited by the application of the available amidation chemistries that were used to selectively incorporate the functionalities. One drawback of this approach is the requirement of a primary amine group on the desired functionality to enable the introduction of functional groups into the nanoparticles. Of further concern is that the amidation chemistries involved may not be compatible with the other functionality present on the reactive group. Additionally, this strategy requires the consumption of a large number of the hydrophilic carboxylic acid groups by cross-linking and functionalisation reactions, which may alter the hydrophilicity of the outer shell layer and result in detrimental effects on the nanoparticle stability and structure.

This two-step, cross-linking/functionalisation strategy can also be modified by combination into a single step by employment of a multi-functional dendritic cross-linker to coincidentally stabilise the supramolecular polymer assembly and embed into the shell unique chemical functionalities (Fig. 13).[42] In the previously reported method, the shell cross-linking of the micelle was performed with the carbodiimide-mediated amidation reaction to afford the 'Click'-readied nanoparticles, which were reacted subsequently with complementary 'Click'-readied small molecules to afford the functionalised nanoparticles. This new strategy involves the direct reaction between 'Click'-readied functional groups in the micelle shell and the 'Click'-readied termini of a dendrimer to perform the cross-linking effectively throughout the shell

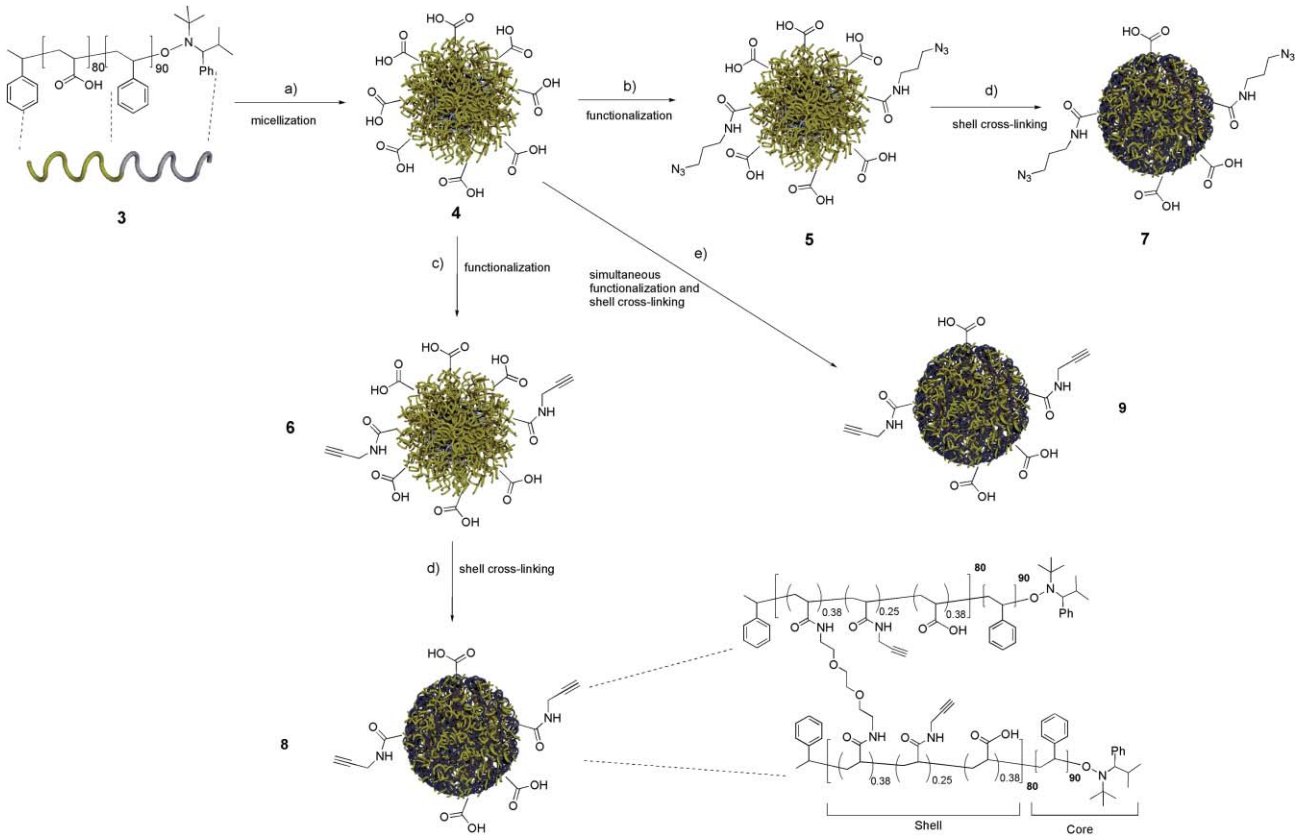

Fig. 12 Synthesis of the shell functionalised 'Click' micelles and nanoparticles using amidation functionalisation and cross-linking strategies. (Reproduced with permission from Ref. 41. Copyright 2005 American Chemical Society.)

layer. The unique application of the polyvalent dendritic cross-linker enables successful stabilisation of the micelle whilst simultaneously incorporating excess functionality that can undergo further complementary functionalisation reactions. Following cross-linking, the remaining unreacted dendrimer chain ends were then reacted quantitatively with azido and alkynyl fluorescently active molecules, which were envisaged as models for Click-readied small molecules, such as biologically-active ligands that could be attached to the nanoparticles. However, in this report only the application of the azido-terminated first generation dendrimer was shown to behave as an effective cross-linker for the polymer micelle shell domain. This was attributed to the incompatibility of the relatively hydrophobic higher generation dendrimers with the aqueous corona domain. This new type of nanoparticle was named shell 'Click' cross-linked nanoparticles (SCCKs) and represent a novel route towards the single step cross-linking and functionalisation of nanoparticles with specific reactive, reporter or targeting groups.

An alternative approach, which has been reported by the group of Yusa,[43] involves the polymerisation of a functional hydrophilic monomer for the incorporation of specific reactive groups within the shell layer. This strategy allows for complete incorporation of the desired functionality into the shell layer

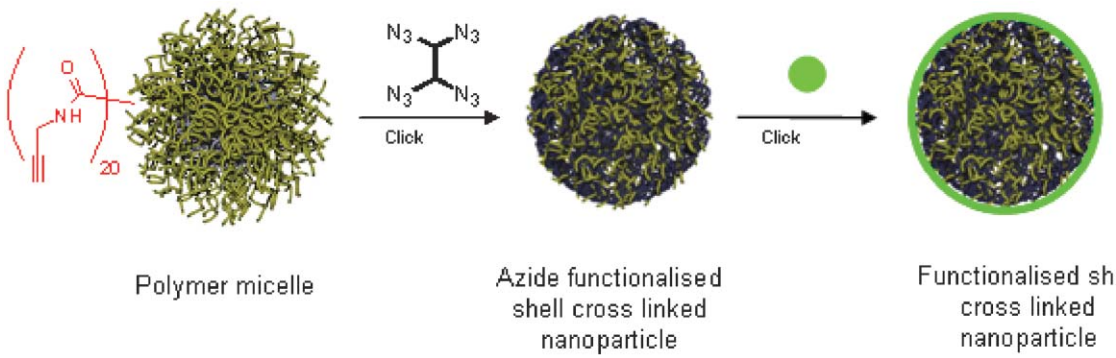

Fig. 13 Schematic representing the simultaneous cross-linking and functionalisation of an alkynyl functionalised micelle by reaction with a G1-azido terminated dendrimer under 'Click' conditions. (Reproduced with permission from Ref. 42. Copyright 2005 American Chemical Society.)

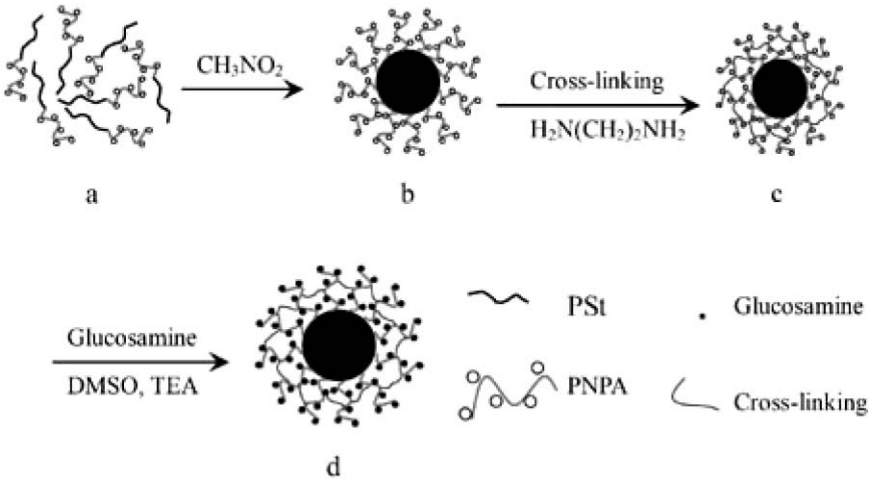

Fig. 14 Formation of stable gluosamine functionalised nanoparticles from the diblock copolymer poly(*p*-nitrophenyl acrylate)-*b*-poly(styrene). (Reproduced with permission from Ref. 44. Copyright 2005 American Chemical Society.)

and has been successfully demonstrated for phospholipids by utilising 2-methacryloyloxyethylphosphoryl choline as a monomer and CRP techniques. However, micelles prepared *via* this methodology may not be capable of undergoing shell cross-linking, due to a lack of suitable reactive groups, to afford a stabilised robust nanoparticle. A more versatile methodology may involve the random copolymerisation of the active functional monomer with a non-functionalised monomer to allow for tailoring of the degree of functionalisation, whilst still enabling cross-linking of the shell layer.

A second approach was recently reported by Pan[44] for the synthesis of glucosamine functionalised nanoparticles (Fig. 14). This approach involves the initial synthesis of micelles furnished with glucosamine functionality by the reaction of a reactive shell poly(*p*-nitrophenyl acrylate)-*b*-styrene with the desired functionality (glucosamine) to give an amphiphilic structure which can undergo self assembly followed by a second shell cross-linking step. Interestingly the same chemistry was used for the cross-linking and functionalisation of the nanoparticles and was based on the reaction of a nitrophenyl group with either a primary diamine cross-linker or glucosamine. These corona functionalised cross-linked micelles were demonstrated to have specific interactions with Concanavalin A by analysis of the precipitation of the glycopolymers micelles, which was monitored by turbidity measurements.

Given the wealth of chemistries available in the shell layer, it is important to note that any micelle with a reactive (*e.g.* hydroxyl, pyridyl, carboxylic acid, nitro, amino, *etc.*) hydrophilic monomer can be chemically modified to incorporate reactive functionality, thus the scope of micelle and nanoparticle shell functionalisation is vast. For example, Armes[21] recently reported shell cross-linked micelles with hydroxyl functionalised coronas and compared them to dendrimers. Similarities have previously been made between dendrimers and stabilised micelles as they both are nanosized globular structures with three distinct domains and controllable functionality. Although dendrimers are generally more time consuming to prepare than are micelles, their main advantage is their very large number of surface functional groups in combination with their well-defined macromolecular architecture. To mimic the multiple functionality displayed by dendrimers, the authors utilised a self-assembled triblock copolymer in which the central amino block could be cross-linked and the peripheral block was a glycerol monomethacrylate, which presented reactive functionality. This strategy afforded a shell of reactive hydroxyl groups, surrounding the shell cross-linked nanoparticle.

Core functionalisation

The selective functionalisation of the core domains of polymer micelles is an exciting area of research, due to the potential application of the resulting materials as novel carrier systems. The covalent attachment of specific groups within the hydrophobic core, which can be protected from hydrolysis or degradation by the surrounding hydrophilic corona, allows for the delivery of hydrophobic moieties in a hydrophilic or physiological environment. However, the functionalisation of the core domain within polymer micelles and nanoparticles has received limited attention, perhaps due to the difficulty in incorporating and retaining functionality within the hydrophobic block. However, recent developments in the synthesis of acid sensitive copolymer micelles by Fréchet have demonstrated the successful incorporation of cleavable hydrophobic functionality within the core domain and their use in acid sensitive delivery systems.[45]

Katakota has also reported the preparation of drug carriers with pH-triggered drug release, using an amphiphilic diblock copolymer of poly(ethylene)glycol-*b*-poly(aspartate hydrozone adriamycin) (Fig. 15).[46] By utilising this drug functionalised monomer containing an acid sensitive linker between the drug and the polymer backbone, it was possible to synthesise the core functionalised micelle, for specific release. This was demonstrated by an observed dynamic change in the micelles in response to environmental stimuli. For example, a change in pH triggered the release of the core loaded drug molecule ADR and its release could be monitored by fluorescence spectroscopy which confirmed the localisation of the ADR in the cell nuclei. A drawback of this methodology is that

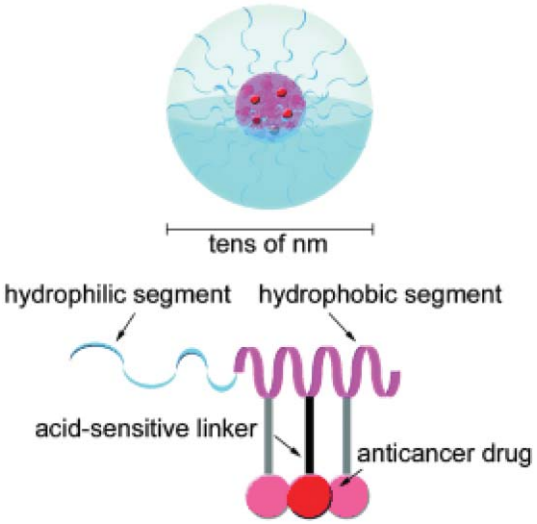

Fig. 15 Schematic illustrating the polymer micelles prepared from the self assembly of amphiphilic block copolymers containing a functional monomer incorporating an acid sensitive linker and an active drug moiety. (Reproduced with permission from Ref. 46. Copyright 2003 Wiley-VCH.)

relatively complicated synthetic procedures were required for the diblock copolymer synthesis as the reactive functionality is not compatible with the required polymerisation conditions.

Another strategy that is perhaps more synthetically versatile and straightforward is the synthesis of latent functionalised monomers, which can be incorporated selectively into the hydrophobic domain to allow for the selective and versatile functionalisation of the core region. Using 'Click' reactive groups as the latent functionality, these Click-functionalised micelles were cross-linked in an intramicellar fashion *via* amidation reactions within the shell layer to afford nanoparticles bearing alkynyl or azido groups within the core domains (Fig. 16).[41,47] The azido or alkynyl functionality that was embedded and dispersed throughout the hydrophobic styrenic core of the polymer micelles was demonstrated to be available for further chemical modification using 'Click' chemistry with complementary 'Click'-functionalised fluorescent dyes, thus allowing the versatile chemical modification of the core domain.

Functionalisation of the nanoparticle core requires that a number of issues, not present for the shell functionalisation, be considered. Unlike the shell, which is a swollen hydrogel, the core is hydrophobic and for covalent attachment to occur

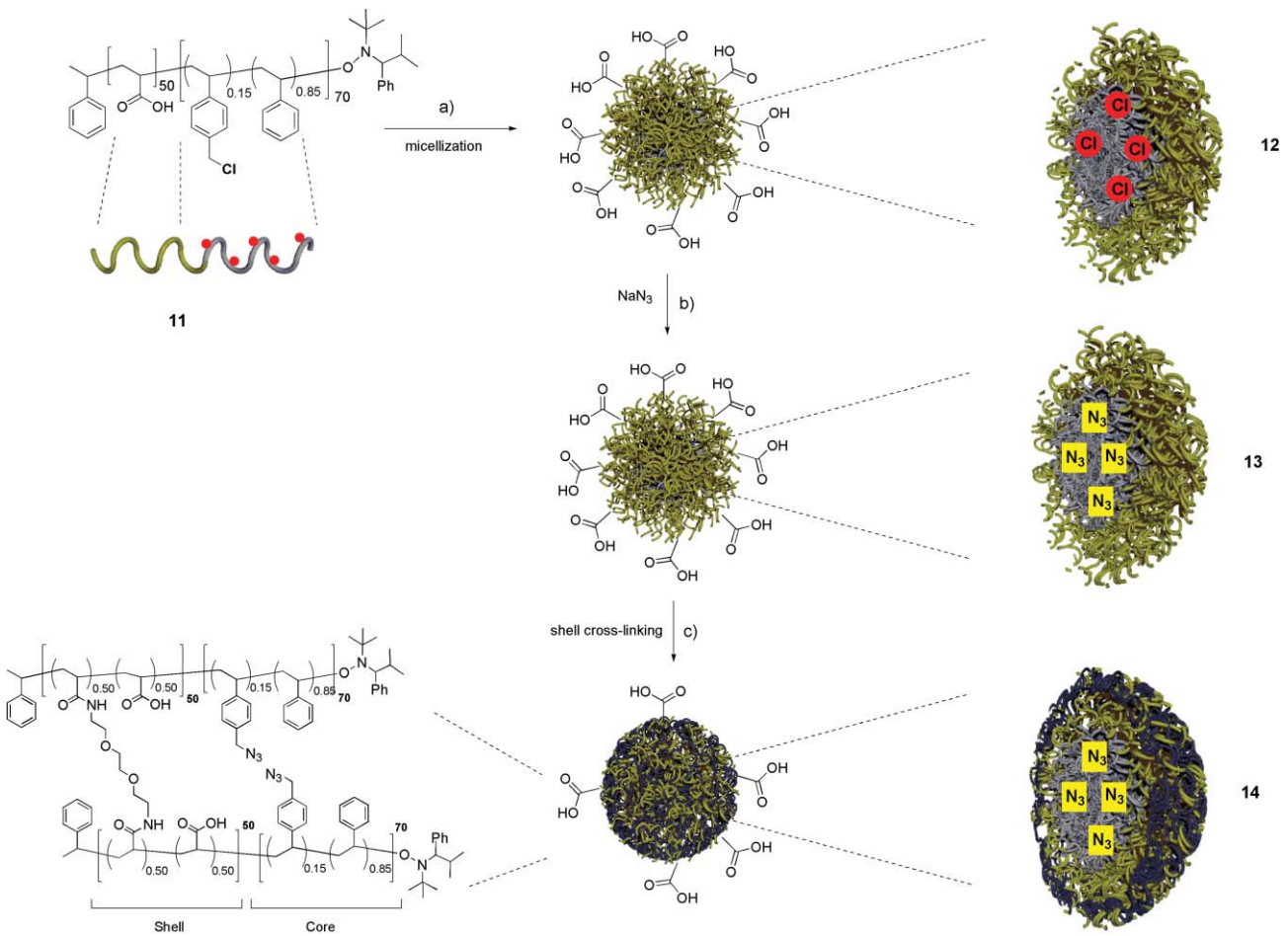

Fig. 16 Schematic for the synthesis of core azido functionalised micelles, using chloromethyl styrene as a latent functional monomer. (Reproduced with permission from Ref. 41. Copyright 2005 American Chemical Society.)

within this domain, reagents and substrates must transverse the shell layer and core–shell interface while still being soluble in the hydrophobic nano-environment of the core. These multiple issues were addressed and it was reported that the 'Click' reaction conditions and dye molecule employed for the cycloaddition could be tailored to match the environment when the functionalisation was located within the nanoparticle core domain. When using these tailored conditions, the covalent attachment *versus* physical sequestration of a fluorescein fluorophore was confirmed using analytical ultra-centrifugation analysis and also utilisation of a pro-fluorophore coumarin dye.

It has been demonstrated that a fluorescent signal could be triggered in this molecule by the 'Click' reaction upon formation of a triazole ring between the azidocoumarin and alkynyl functionalised small molecule, due to the resulting alteration of the electronic structure.[48] Utilising this principle, it was demonstrated that the fluorogenic reaction between a 3-azidocoumairn and alkynyl functionality located within the hydrophobic core domain of a shell cross-linked nanoparticle was successful, based on the generation of fluorescence upon alteration of the coumarin electronic structure. This chemistry allowed for confirmation of the covalent 'Click' attachment of the 3-azidocoumarin within the nanoparticle.

Concluding remarks

Although the self assembly of amphiphilic block copolymers has been an area of great interest for chemists for some time, quite recently the activities in the areas of functional and stabilised polymer micelles have been intensified considerably. The availability and realisation of new synthetic controlled radical polymerisation methods offer the opportunity to synthesise tailor made, well-defined di- and tri-block copolymers, thus providing an increase in synthetic versatility and explain this increase in interest. The current research has enabled the realisation of the full potential of these polymer micelles and the next stage in their development depends on the further design and implementation of facile and versatile strategies for functionalisation and cross-linking, to allow for the selective tailoring of the materials towards specific applications. Due to the complexity of these functionalised systems, an important aspect of their development is the further investigation into the availability of reactive groups within various domains of the nanostructures. An interesting target in the functionalisation of these materials involves the orthogonal introduction of different functionalities within different locations within a single nanoparticle to try to mimic and achieve some of the structural complexity observed in natural systems.

One disadvantage of these polymer micelle systems is the scale up of the majority of the current techniques utilised to synthesize these materials is extremely difficult. This will impact their commercial exploitation and thus potential applications which are proposed to be currently limited to high end applications. Thus significant work in the field of polymer micelles should be directed towards the more efficient, less costly and time consuming synthesis of these functionalised materials. The development of new scalable chemistries for the synthesis and the discovery of more generic synthetic routes will significantly advance the potential and application of these nanoscale materials.

In particular, block copolymer micelles and nanoparticles hold great promise for drug delivery applications, primarily due to their distinct core–shell morphology and potential for functionalisation. However, recent interest has focused on the application of polymer micelles as nanoscale reaction vessels in which to perform chemistries.[49] A major advantage of using such nanoscale constructs as reaction vessels includes the concentration of reactants within the core domain and also the possibility of performing chemistry within a confined reaction environment. In addition, the application of diblock copolymers containing a poly(acrylonitrile) segment has been elegantly utilised as a scaffold for the preparation of well-defined nanoscale carbon nanoparticles. This was achieved by the utilisation of conventional diblock copolymer micellisation and shell cross-linking techniques followed by subsequent removal of a sacrificial poly(acrylic acid) domain to afford well-defined nanoscale carbon particles.[50] Interest is growing in the application of new cross-linking and functionalisation strategies for these polymer assemblies to enable their potential in advanced applications. Moreover, iterative and repetitive sequences of supramolecularly-directed assembly and covalent chemistry are allowing for combined physical and chemical manipulation of the nanoscale objects to increase their levels of complexity and sophistication, while maintaining simplicity in the processes.

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