Ring-Opening Polymerization of *N*-Carboxyanhydrides for Preparation of Polypeptides and Polypeptide-Based Hybrid Materials with Various Molecular Architectures

David Pahovnik and Nikos Hadjichristidis

Abstract Different synthetic approaches utilizing ring-opening polymerization of *N*-carboxyanhydrides for preparation of polypeptide and polypeptide-based hybrid materials with various molecular architectures are described. An overview of polymerization mechanisms using conventional (various amines) as well as some recently developed initiators (hexamethyldisilazane, *N*-heterocyclic persistent carbenes, etc.) is presented, and their benefits and drawbacks for preparation of polypeptides with well-defined chain lengths and chain-end functionality are discussed. Recent examples from literature are used to illustrate different possibilities for synthesis of pure polypeptide materials with different molecular architectures bearing various functional groups, which are introduced either by modification of amino acids, before they are transformed into corresponding *N*-carboxyanhydrides, or by post-polymerization modifications using protective groups and/or orthogonal functional groups. Different approaches for preparation of polypeptide-based hybrid materials are discussed as well using examples from recent literature. Syntheses of simple block copolymers or copolymers with more complex molecular architectures (graft and star copolymers) as well as modifications of nanoparticles and other surfaces with polypeptides are described.

Keywords Polypeptides • *N*-carboxyanhydrides • Ring-opening polymerization • Hybrid materials • Molecular architectures

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1 Introduction

Polypeptide materials are of great interest due to the very broad functionalities that can be introduced into polypeptide chain as well as due to their ability to form different secondary structures (α-helix, β-sheets, etc.), which play a very important role in their self-assembly and have thus consequently a very significant effect on their chemical and mechanical properties. Recent developments in the field of polypeptide synthesis by ring-opening polymerization (ROP) of α-amino acid *N*-carboxyanhydrides (NCA) enabled us preparation of well-defined polypeptides with different molecular architectures. When polypeptides are combined with other polymers, hybrid materials are prepared, which combine the functionality and ability of self-organization of polypeptides with properties of more conventional polymers, this expanding the possible applications even further. Recently, there have been a number of excellent reviews published on the synthetic polypeptides and polypeptoids prepared by ROP of NCA and their potential applications focusing mainly on the mechanisms and synthesis of complex macromolecular architectures [1, 2], biomedical applications [3, 4], stimuli responsive polypeptides [5] and their self-assembly [6], or polypeptoids [7]. In this work we will present a general overview of a very fast developing field of synthetic polypeptides with regard to the mechanism of polymerization using different conventional as well as some more recently developed initiators for preparation of polypeptides with well-defined chain lengths and chain-end functionality. Using examples from recent literature, different possibilities for synthesis of pure polypeptide materials as well as polypeptide-based hybrid materials with different molecular architectures are described.

2 Polymerization Mechanisms

Due to the structure of the NCA molecule, bearing multiple possible sites for either nucleophilic or electrophilic attack (Fig. 1), there are several possible initiating systems that can be used for the ROP of NCAs, each following their own mechanism having various advantages as well as disadvantages.

High reactivity of the NCA monomers comes hand in hand with the necessity to have them as pure as possible, so that we are able to prepare well-defined polypeptides and avoid a large number of possible side reactions. In most cases, repeated crystallization is enough to obtain pure solid NCAs. On the other hand, oily NCAs can be purified by washing the reaction mixture with water and sodium bicarbonate [8]. Washing also proved efficient in cases where acid scavengers (triethylamine) had to be used with acid-labile protective groups on the amino acid [9]. However, washing has to be done at low temperature to avoid hydrolysis of NCAs, and water has to be completely removed afterwards since it can act as initiator as well. Another option for purification of NCAs is flash column

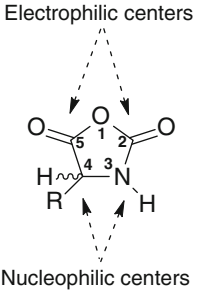

Fig. 1 NCA with possible reaction centers shown

chromatography on silica. This procedure is especially useful for the preparation of highly functional and low-melting NCAs that are difficult to crystallize. However, it has to be done under anhydrous conditions in a glove box [10]. To ensure not only high purity of all reagents involved but also maintain the necessary conditions for the living polymerization of NCAs, high-vacuum technique for purification of the NCAs by crystallization and subsequent polymerization was developed [11]. The NCA monomer was introduced into a glass apparatus specially designed for crystallization (Fig. 2), which was then attached to the high-vacuum line. High molecular weight homopolypeptides with very low dispersities were obtained using primary amine initiators, and the livingness of the chains was confirmed by subsequent addition of other monomers to make diblock and triblock copolymer, which also had very low dispersities and expected molecular weights. The livingness of the polypeptides under high-vacuum conditions can be preserved indefinitely, thus making possible the use of linking chemistry for the synthesis of a wide variety of macromolecular architectures. The high-vacuum technique for purification of NCAs and subsequent polymerization was shown to be very effective for preparation of highly reactive NCAs, e.g., L-prolin and well-defined poly(L-prolin) homopolypeptides as well as diblock and triblock hybrids with PEO, where poly (L-prolin) forms a hydrophilic helix independent of pH and temperature within the physiological range [12].

The most commonly used initiators for the NCA polymerization are primary amines, which follow the normal amine mechanism (NAM) of polymerization. The amino group attacks the carbonyl atom at position 5, which is followed by the opening of the ring, yielding a peptide bond and carbamic acid. Carbamic acid then undergoes decarboxylation to give a free primary amino group, which acts as a new initiator, thus enabling the chain growth (Fig. 3) [13, 14]. Other nonionic initiators like secondary amines, alcohols, water, etc., can follow the same mechanism, as long as they have a labile proton bound to the nucleophilic center.

If instead of a nucleophilic amine, we use a nonprotic base such as triethylamine, it will deprotonate the NCA by taking away the proton at the 3-N. This creates an NCA anion which then acts as an initiator, so this mechanism is called activated monomer mechanism (AMM) [15]. Since a proton is required at the position 3-N, this mechanism only works for *N*-unsubstituted NCAs. The NCA anion attacks the 5-carbonyl group of another NCA molecule opening the ring. The carbamate anion

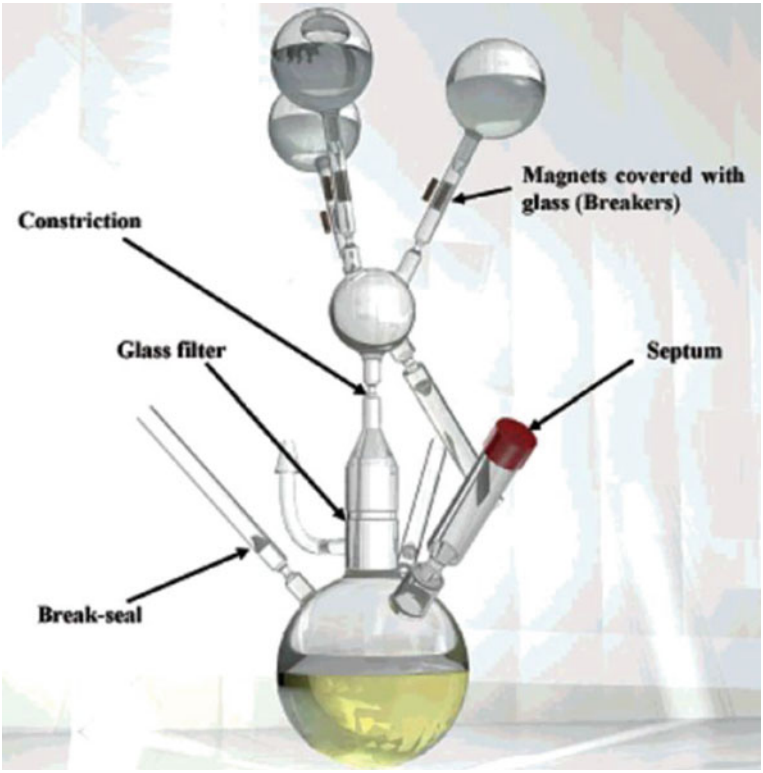

Fig. 2 Apparatus used for purification of NCAs by high-vacuum technique (Reprinted with permission from Aliferis et al. [11]. Copyright 2004 American Chemical Society)

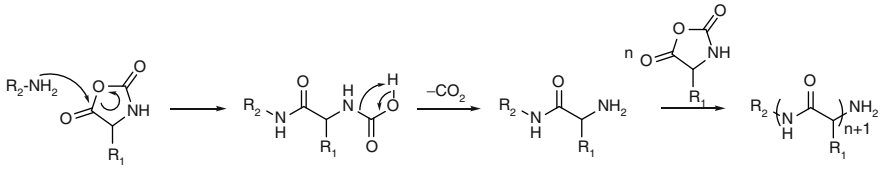

Fig. 3 Normal amine mechanism of NCA polymerization

then deprotonates a new NCA which forms an NCA anion, while the carbamic acid decarboxylates to give an amino group. The formed dimer now has two reactive centers capable of propagation. The new NCA anion can attack the 5-carbonyl group of the dimer, and a new carbamate is formed which deprotonates another NCA so it can decarboxylate, thus regenerating the NCA anion needed for propagation, which can however still act as a new initiator thus leading to poorly controlled polymerization. On the other side, the amino group can react with NCA to propagate via NAM (Fig. 4). Since most nucleophiles can act as a base,

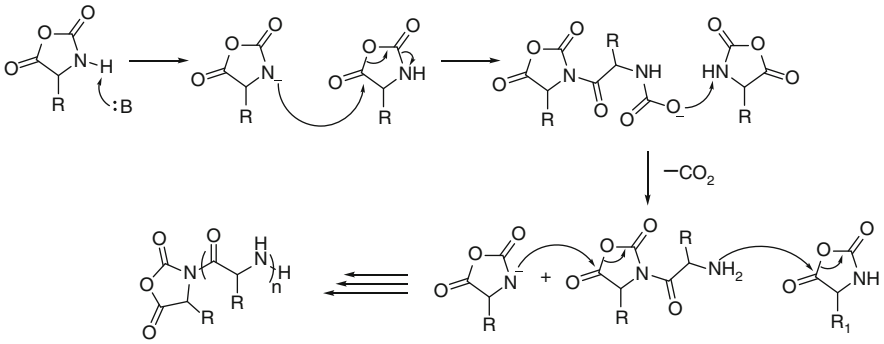

Fig. 4 Activated monomer mechanism of NCA polymerization

both NAM and AMM can coexist. This is especially true for sterically hindered secondary amines, which are stronger bases than the primary amines, and can deprotonate the NCA while still having the proton on the nucleophile so they can undergo the NAM.

To avoid the AMM when using primary amines, use of primary amine salts was proposed. The amine salt can neither initiate nor propagate the polymerization; however, at high temperatures the exchange between the free amino group (active) and protonated one (dormant) becomes faster, making it possible for the NCA to polymerize. The constant presence of protons in the reaction mixture ensures that the NCA stays protonated thus avoiding the AMM. Dimitrov and Schlaad [16] have used a polystyrene macroinitiator with primary amine hydrochloride end group as an initiator. The polymerization was run at 40–80 °C for 3 days. Polypeptides with significantly narrower molecular weight distributions were obtained as compared to polypeptide initiated by primary amine macroinitiator. Since chloride anion is known to be able to initiate the polymerization of NCA, a primary amine salt with a non-nucleophilic anion was used by Vicent et al. [17]. They have prepared a series of primary amine tetrafluoroborate salts ($R\text{-}NH_3^+\ BF_4^-$) and tested them as initiators. They have managed to control the molecular weight of polymers up to a degree of polymerization of about 800 without the use of complex initiators or a demanding experimental setup. The derived polymers had low dispersities, below 1.2.

A special case of secondary amine used for the controlled polymerization of NCA was hexamethyldisilazane (HDMS) [18]. It was expected that HDMS, as a secondary amine with two bulky groups, will react by the AMM; however, it turns out that it follows a unique mechanism. HMDS, as expected, first deprotonates the NCA; however, a trimethylsilyl (TMS) group gets transferred to 2-CO from HMDS, and an intermediate is formed, which is rapidly attacked by the in situ-generated TMS amine causing ring to open, thus forming a TMS carbamate. The polypeptide chain then propagates through the transfer of the TMS group from the terminal TMS carbamate to the incoming monomer to form a new TMS carbamate terminal propagating group (Fig. 5). This mechanism is similar to the group transfer

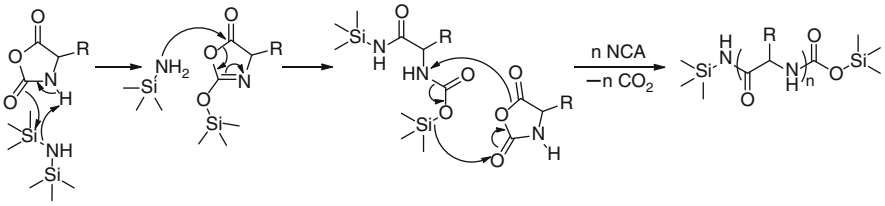

Fig. 5 Proposed mechanism of NCA polymerization using hexamethyldisilazane (HMDS)

polymerizations of acrylic monomers initiated by similar organosilicon compounds. Using HDMS polymerizations proceeded smoothly to yield PBLGs with good control over molecular weight and molecular weight distributions. Other *N*-TMS amines can be used as initiators as well, enabling functionalization at the C-terminus [19].

Deming [20–22] moved away from the amine-based initiators and developed a new class of initiators for NCA polymerization based on transition metal complexes of cobalt and nickel. By using the zerovalent nickel or cobalt complexes with the right ligands (2,2′-bipyridyl (bipy), 1,5-cyclooctadiene (COD), and trimethylphosphine (PMe_3), he was able to prepare well-defined polypeptides. In the proposed mechanism [23], the metal reacts with the NCA by oxidative addition to the anhydride group, forming a metallacyclic complex, which reacts with another NCA to give a 6-membered cyclic intermediate. This intermediate upon further reaction with NCA followed by a ring contraction produce a 5-membered amido-amidate metallacycle, which is the active polymerization intermediate, through which the polymerization then proceeds (Fig. 6). The obtained polypeptides had narrow molecular weight distributions ($Đ_M < 1.3$), and good control over the molecular weight was achieved. However, this type of initiators does not work for the polymerization of *N*-substituted NCAs.

Besides cobalt and nickel, a number of other transition elements have proven to be efficient initiators for NCA polymerization, including platinum, where it was again shown that the choice of the right chelating groups plays the most important role in the activity of the metal complex [24]. It also determines the efficiency of the metal complex to keep the NCA polymerization living and to give polypeptides with narrow molecular weight distributions.

Recently, *N*-heterocyclic persistent carbenes (NHC) have been successfully used as initiators for polymerization of *N*-substituted NCAs to synthesize poly(α-peptoid)s [25]. This mechanism does not work for *N*-unsubstituted NCAs, since imidazolium-based persistent carbenes are not only good nucleophiles but are a strong base as well, easily deprotonating the *N*-unsubstituted NCAs. They are actually capable of deprotonating the methylene group of the monomer, which leads by yet unknown mechanism to the formation of a proposed Münchnone zwitterion-initiating species. This species is supposed to have an exocyclic carbamate group from which subsequent monomer addition may occur (Fig. 7a). NHC-mediated ring-opening polymerization can proceed through another

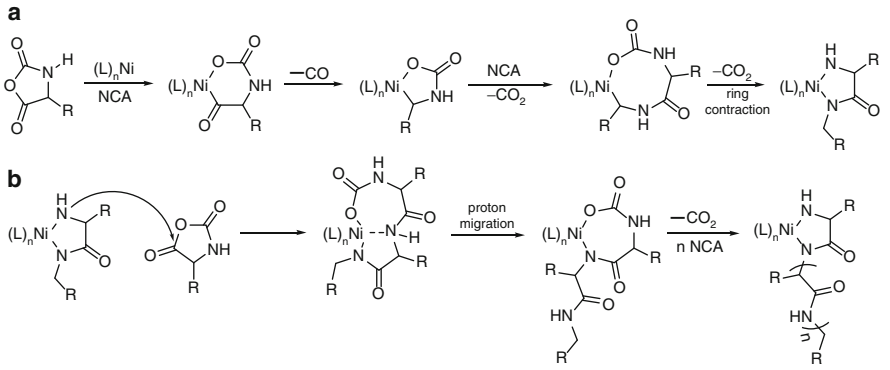

Fig. 6 Proposed mechanism of NCA polymerization using zerovalent nickel complex. Path (**a**) represents the creation of the initiating species, while path (**b**) shows the mechanism of propagation

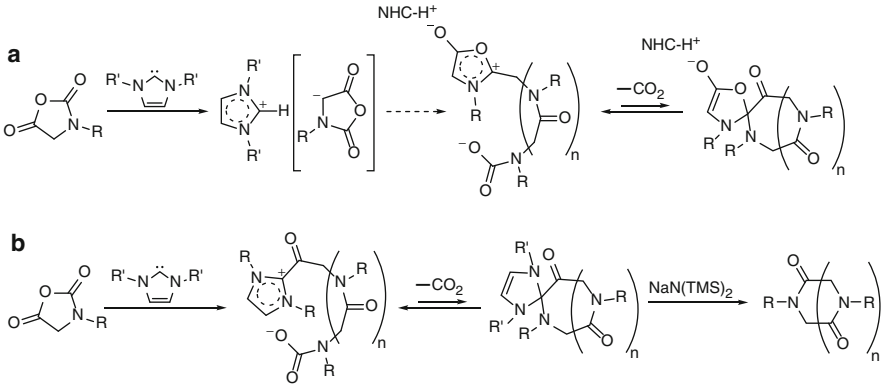

Fig. 7 Proposed mechanisms for NHC-mediated ring-opening polymerization of *N*-substituted NCAs proceeding through deprotonation of NCA (**a**) or nucleophilic attack of NHC on NCA (**b**) with formation of the spirocycles and subsequent cyclization with $NaN(TMS)_2$ to give cyclic poly (α-peptoid)s

mechanism, where after the attack of the carbene and ring opening of the NCA, a zwitterionic intermediate is formed that transforms into spirocyclic product, which upon treatment with $NaN(TMS)_2$, is turned into a free cyclic poly(α-peptoid) (Fig. 7b) [26]. The control over the polymer molecular weight strongly depends on the solvents and the NHC structure, since only low molecular weight polymers were obtained regardless of the initial monomer/initiator ratio when *N*,*N*-dimethylformamide was used. However, in tetrahydrofuran or toluene, very low dispersities were obtained ($Đ_M < 1.12$) even for high molecular weights with good control over the molecular weight. In solvents with low dielectric constant, zwitterionic intermediate species maintain cyclic architectures with two chain ends in close contact

through Coulombic interaction, which significantly suppresses the side reactions. The livingness of the system was tested with chain-extension experiment, and an increase of polymer molecular weight was observed with no low molecular weight species formed [25].

3 Polypeptide Materials with Various Molecular Architectures

Due to the large selection of possible amino acids and their readiness for further modifications, various different NCA monomers were successfully polymerized. A broad selection of protective groups for the side chains have been used that enable selective deprotection and post-polymerization modifications to afford polypeptides with desired functionality. A high grafting efficiency can usually be achieved, especially in the case of polypeptides that form α-helix since it can reduce steric hindrance and result in increased accessibility of the side chains for reaction [27]. Another method is to modify the amino acid prior to the cyclization to prepare the NCAs. Completely modified polypeptides can be prepared this way; however, complex substituents often require exotic protective groups and/or reaction conditions to survive the formation of the NCA as well as purification can be significantly harder, especially if the substituted NCAs do not crystallize.

Preparation of various well-defined biomimetic analogs shows a lot of promise for biomedical applications. Glycopolypeptides were so prepared by a transformation of ε-amino group on lysine to azido group using a diazotransfer reagent imidazole-1-sulfonyl-azide · HCl [28]. Modified amino acid was then cyclized to NCA, and homo- and copolymers with Cbz-lysine using $(PMe_3)_4Co$ initiator were prepared with narrow molecular weight distributions (Fig. 8). The azido functional polypeptides were then readily modified with alkyne functional glycoside by copper-catalyzed azide−alkyne cycloaddition to afford glycopolypeptides.

Formation of various secondary structures (α-helix, β-sheets, etc.) of the polypeptide chains plays a very important role in their self-assembly and have consequently a very significant effect on their chemical and mechanical properties. Hammond et al. [29] developed a set of hydrogels based on poly(-γ-propargylglutamate). Homopolymer was then grafted with diethylene glycol using copper-catalyzed azide–alkyne cycloaddition. Diethylene glycol grafted poly(γ-propargylglutamate) with only L-amino acids exists in predominantly α-helix conformation, while polypeptides prepared from a racemic mixture of amino acids adopt random coil conformation. To prepare the hydrogels, hydroxyl groups of diethylene glycol grafted to poly(γ-propargylglutamate) were reacted with 4-(maleimido)phenyl-isocyanate to transform them to maleimide functionality, followed by cross-linking with a thiol-functionalized poly(ethylene oxide) (PEO) star (Fig. 9). Both hydrogels with either helical or coil polypeptides exhibited similar swelling and permeability, but dramatically different stiffness due to rigidity of the polypeptide backbone.

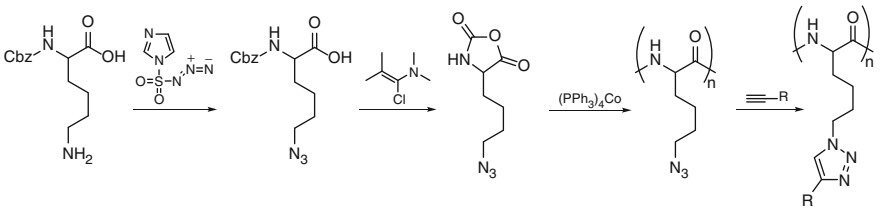

Fig. 8 Modification of lysine to prepare azide-containing polypeptides

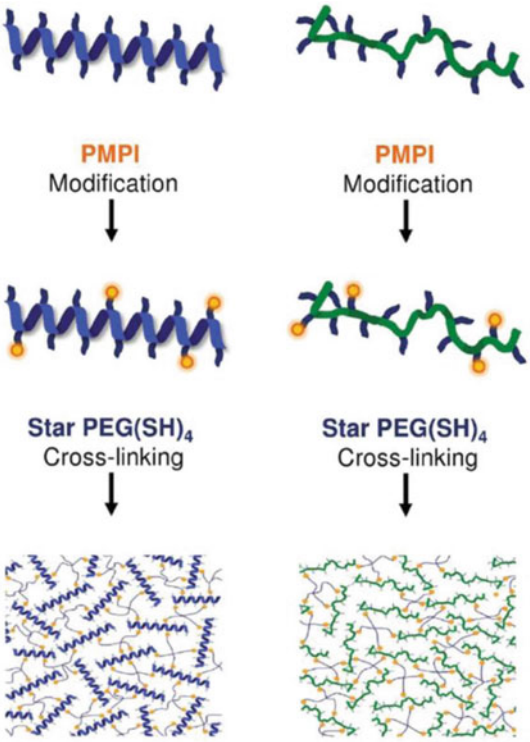

Fig. 9 Preparation of hydrogels with either helical or coil polypeptides with the same formulation (Reproduced from Oelker et al. [29] with permission of The Royal Society of Chemistry)

Glycopolypeptides were also prepared by Gupta et al. [30]; however, instead of post-polymerization modification, they have attached protected galactose, mannose, or lactose to L-lysine, which was then transformed to NCA, polymerized, and finally deprotected to afford water-soluble glycopolypeptides. Glycopolypeptides prepared from purely L-lysine-modified amino acid adopt an α-helix conformation in water, while glycopolypeptide prepared from racemic mixture of lysine showed no helicity. A self-assembly of such glycopolypeptides was studied and prepared by azide–amine-terminated bifunctional oligopeptide PEO to which a hydrophobic dendron of different generations were attached by copper-catalyzed azide–alkyne cycloaddition. The self-assembly was found to depend not only upon

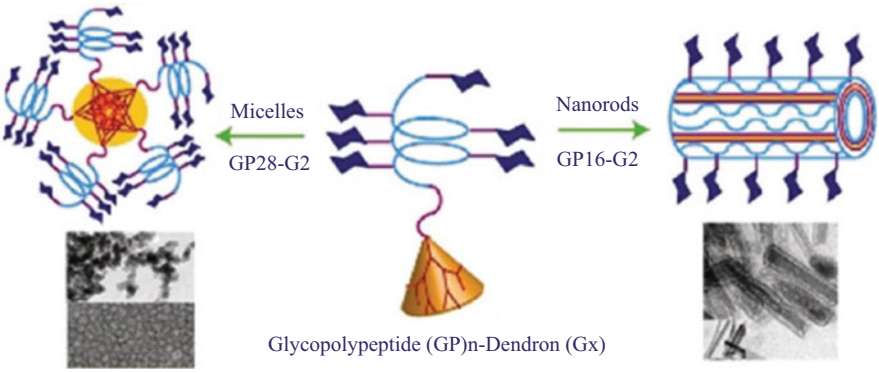

Fig. 10 Self-assembly of a hydrophilic glycopolypeptide chains with a wedge-like hydrophobic dendron attached to one end stiff (Reprinted with permission from Pati et al. [31]. Copyright 2012 American Chemical Society)

generation of the dendron and the length of the glycopeptide segment, but on extent of helicity of the polypeptide backbone as well. A range of one-dimensional to three-dimensional topologies were thus prepared (Fig. 10) [31].

Similarly, Gupta et al. [32] have prepared phosphopolypeptides by attaching a diethylphosphate or diethylphosphonate to L-cysteine thiol group by thiol–ene click reaction. A bifunctional alkyne–amine initiator was used for polymerization of phosphate containing NCAs (Fig. 11). Obtained molecular weights were close to the expected ones, and dispersities below 1.12 were achieved. An azide-functional PEO was then attached to the polypeptides by copper-catalyzed azide−alkyne cycloaddition to afford block copolymers. To make the polypeptides water soluble, the phosphoester groups had to be deprotected. Iodotrimethylsilane was used successfully for deprotection of phosphonate esters; however, in the case of phosphate ester, partial removal of the phosphorus from the polymer chain was unavoidable.

Chan-Park et al. [33] have prepared a series of random co- and terpolymers by polymerization of lysine NCA as hydrophilic and alanine, phenylalanine, or leucine NCAs as hydrophobic amino acids. $Ni(COD)_2$ was used as initiator, and number-average molecular weight of the peptides from around 5 to 35 kDa and narrow molecular weight dispersities of 1.1–1.3 were obtained. The hydrophobic amino acid content was varied from 0 to 100 %. Poly(lysine-*ran*-phenylalanine) and poly (lysine-*ran*-phenylalanine-*ran*-leucine) showed the best antimicrobial properties and even lower minimum inhibitory concentrations than values of many naturally occurring antimicrobial peptides. Optimal antimicrobial activity was achieved when the polypeptide was 25 units long with 60 % hydrophobic amino acid content. No distinct α-helix, β-sheet, or random coil structures were evident in all the peptides indicating that the lack of a distinct secondary structure in poly(lysine-*ran*-phenylalanine-*ran*-leucine) and poly(lysine-*ran*-phenylalanine) did not compromise the peptides' antimicrobial activity.

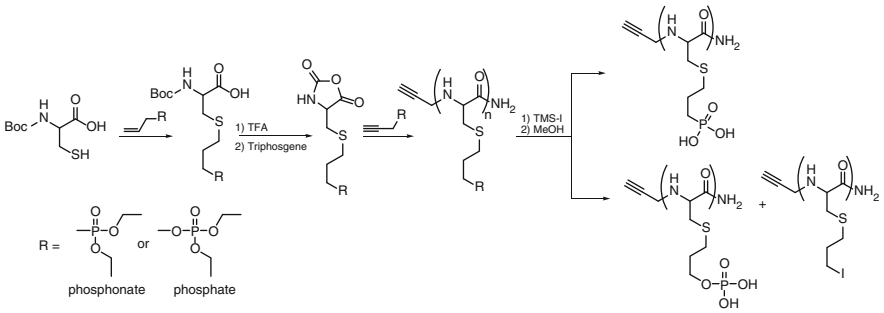

Fig. 11 Synthesis of phosphopolypeptides from L-cysteine

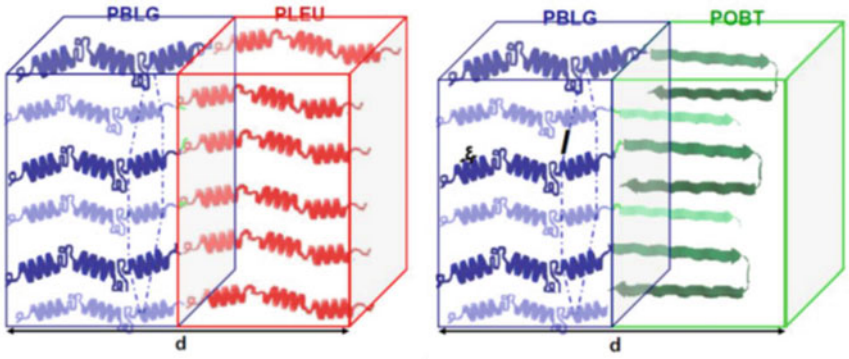

Fig. 12 Schematic representation of the self-assembly in the diblock copolypeptides showing PBLG α-helices that are hexagonally packed and relatively disordered α-helices within the PLEU domain or multiple-folded β-pleated sheets of POBT (Reprinted from Mondeshki et al. [34]. Copyright (2011), with permission from Elsevier)

Floudas et al. [34] prepared two types of poly(γ-benzyl-L-glutamate) (PBLG) diblock copolypeptides with either poly(L-leucine) (PLEU) or poly(*O*-benzyl-L-tyrosine) (POBT) as the second block. NCAs were purified by high-vacuum technique and polymerization was done using *n*-hexylamine as initiator. The first copolymer consists of two blocks that both form α-helix secondary structure, while the POBT block in the second copolymer forms β-strands. After the self-assembly of the diblock copolypeptides, it turns out that the nanoscale confinement in the copolymers preserves the main secondary motifs but affects their folding and lateral packing: β-pleated sheets are multiple folded, whereas the PBLG and PLEU α-helices are defected and of limited lateral coherence (Fig. 12).

The self-assembly of a diblock copolymer was studied for the case of poly (γ-benzyl-L-glutamate)-*b*-polyalanine as well. Block copolymers were prepared under high vacuum using *n*-hexylamine as initiator. Floudas et al. [35] discovered that although pure polyalanine (PAla) stabilizes both α-helices and β-sheets, in the

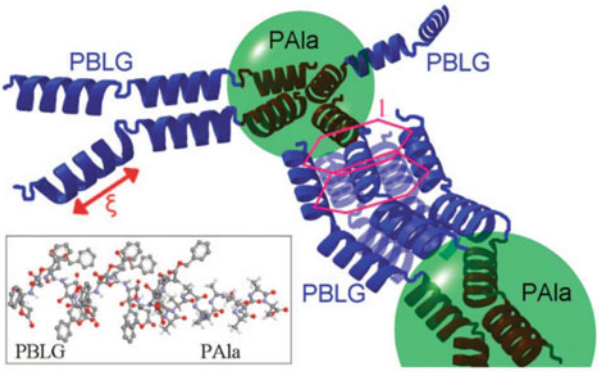

Fig. 13 Schematic representation of the diblock copolymer self-assembly showing PBLG and PAla α-helices that are hexagonally packed (Reprinted with permission from Mondeshki et al. [35]. Copyright 2008 American Chemical Society)

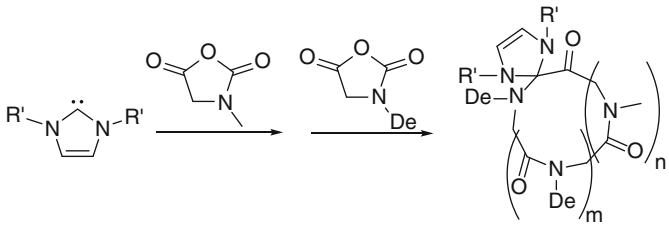

Fig. 14 Synthesis of amphiphilic cyclic diblock copolypeptoids by *N*-heterocyclic carbene-mediated ring-opening polymerization

copolypeptides the latter motif is suppressed due to the strong effects of the thermodynamic field on the peptide secondary structures (Fig. 13), indicating that the PAla β-sheets are less stable than α-helices and hence more prone to the thermodynamic restrictions imposed by the copolymers.

Cyclic block poly(*α*-peptoid)s were prepared by copolymerization of *N*-methyl and *N*-decyl-*N*-carboxyanhydride monomers by NHC-mediated ring-opening polymerization (Fig. 14) [36]. They have shown that adjusting the initial monomer to NHC molar ratio can readily control the block copolymer chain length and composition. A series of amphiphilic cyclic diblock copolypeptoids were thus prepared with variable molecular weight and composition that self-assemble into spherical micelles which reorganize into micrometer-long cylindrical micelles with uniform diameter in room temperature methanol. To prepare cyclic poly(α-peptoid)s that adopt helical conformations, a monomer with chiral side group, (S)/(R)-*N*-phenylethyl *N*-carboxyanhydrides, was polymerized by *N*-heterocyclic carbene-mediated ring-opening polymerization [37]. Polymers with the degree of polymerization up to 95 and low $Đ_M < 1.10$ were obtained; however, attempts to synthesize the polymers of higher molecular weight were unsuccessful.

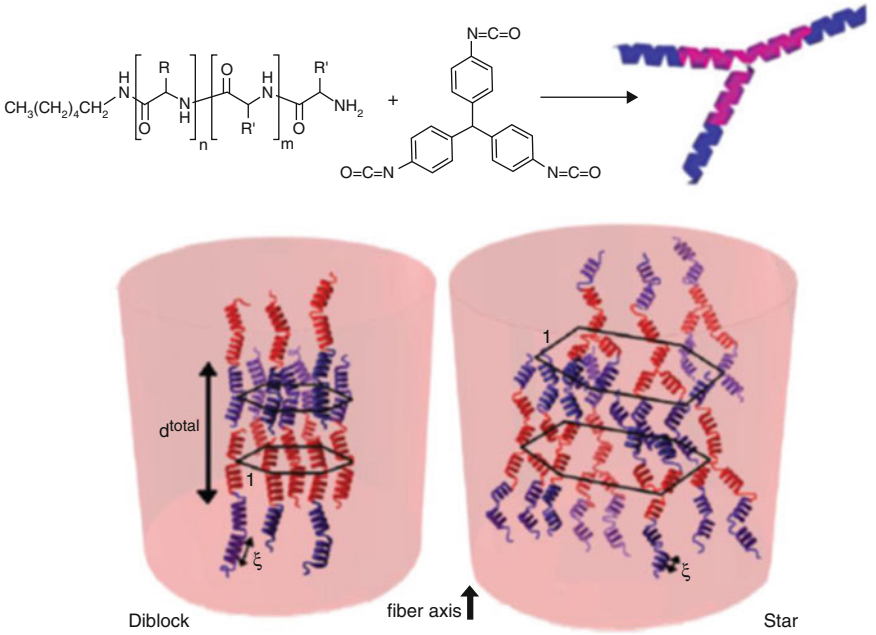

Fig. 15 Synthesis of three-arm star diblock copolymers of γ-benzyl-L-glutamate and ε-benzyloxycarbonyl-L-lysine (*left*) (Reprinted with permission from Aliferis et al. [38]. Copyright © 2005 Wiley Periodicals, Inc.) and a schematic representation of their self-assembly as compared to the diblock copolymers (*right*) (Reprinted with permission from Gitsas et al. [39]. Copyright 2008 American Chemical Society)

Three-arm star homopolymers and diblock copolymers of γ-benzyl-L-glutamate and ε-benzyloxycarbonyl-L-lysine were prepared by Hadjichristidis et al. [38]. The homopolymers and block copolymers were first prepared under high vacuum using *n*-hexylamine as initiator. The star copolymers were prepared by linking the living homo- and block copolypeptides carrying amine end groups with triphenylmethane-4,4′,4″-triisocyanate core (Fig. 15) using high-vacuum technique. For the efficient linking reaction, an excess of the arms was used, which were removed by the salting-out technique to afford pure stars as confirmed by SEC and membrane osmometry showing very narrow molecular weight distribution. Floudas et al. [39] discovered that the chain topology has a very strong influence on the self-assembly of complex copolypeptides. In the diblock copolymers, a lamellar nanostructures were found composed of poly(ε-benzyloxycarbonyl-L-lysine) PZLL and PBLG domains both consisting of α-helical segments that were hexagonally packed. The star topology on the other hand results in mixing of the PBLG and PZLL blocks, which still form α-helices, however, with a smaller persistence length, which are packed in a pseudohexagonal lattice (Fig. 15).

Liu et al. [40] reported on the synthesis of well-defined AB_2 miktoarm polypeptide stars. First, they have polymerized ε-benzyloxycarbonyl-L-lysine from a

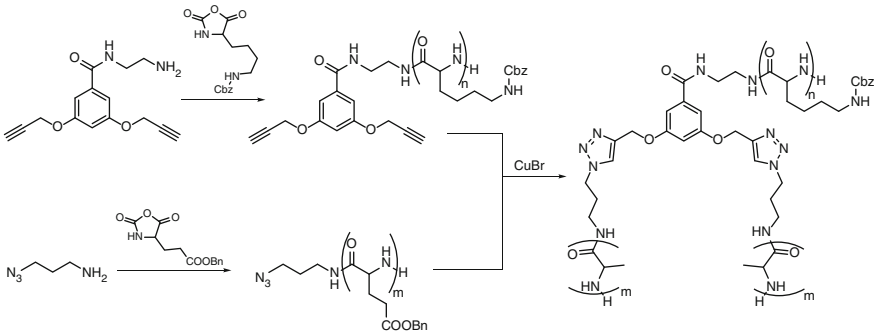

Fig. 16 Preparation of Y-shaped AB_2 miktoarm polypeptide stars

trifunctional initiator, which had one amino group and two alkyne groups and γ-benzyl-L-glutamate NCA from azide–amine difunctional initiator. Coupling of both homopolymers was achieved by copper-catalyzed cycloaddition reaction, and the excess azide-terminated homopolymer was removed by reaction with alkynyl-functionalized Wang resin. Purified miktoarm star copolymer had $Đ_M$ of 1.13; however, molecular weights determined by relative SEC with polystyrene standards were about three times overestimated when compared to the molecular weights determined by NMR. The deprotection of both protective groups was achieved by a one-pot acid hydrolysis to give a pH-responsive Y-shaped miktoarm star polypeptide copolymer (Fig. 16), which self-assemble into poly(L-glutamic acid) (PLGA)-core micelles at acidic pH and poly(L-lysine) (PLL)-core micelles at alkaline pH, accompanied with the coil-to-helix transition of PLGA and PLL sequences, respectively.

Heise et al. [41] prepared a series of star polypeptides based on poly(L-glutamic acid) using polypropylene imine dendrimers of various generations as initiators with a maximum number of arms from 8 to 64. By changing the dendrimer generation as well as the ratio of NCA/amino groups, they were able to prepare a broad range of different star polypeptides (Fig. 17). However, no details about the initiation efficiency were presented to show how many polypeptide arms there really are per dendrimer. SEC traces showed a lower molecular weight shoulder indicating a formation of linear homopolymer through activated monomer mechanism due to the tertiary amines in the dendrimer core. Star copolymers have been tested as drug delivery systems by using rhodamine B, and successful enzyme-responsive release was achieved. The same types of stars have been prepared with poly(L-lysine) to afford cationic star polymers which were tested as gene delivery vectors [42]. The complexation of siRNA as well as transfection of pDNA with star-shaped poly(L-lysine) was greatly superior as compared to linear poly (L-lysine).

Qiao et al. [43] prepared core cross-linked star polypeptides by sequential polymerization of NCAs with di-NCA L-cystine that acts as a cross-linker. γ-Benzyl-L-glutamate or ε-benzyloxycarbonyl-L-lysine was first polymerized

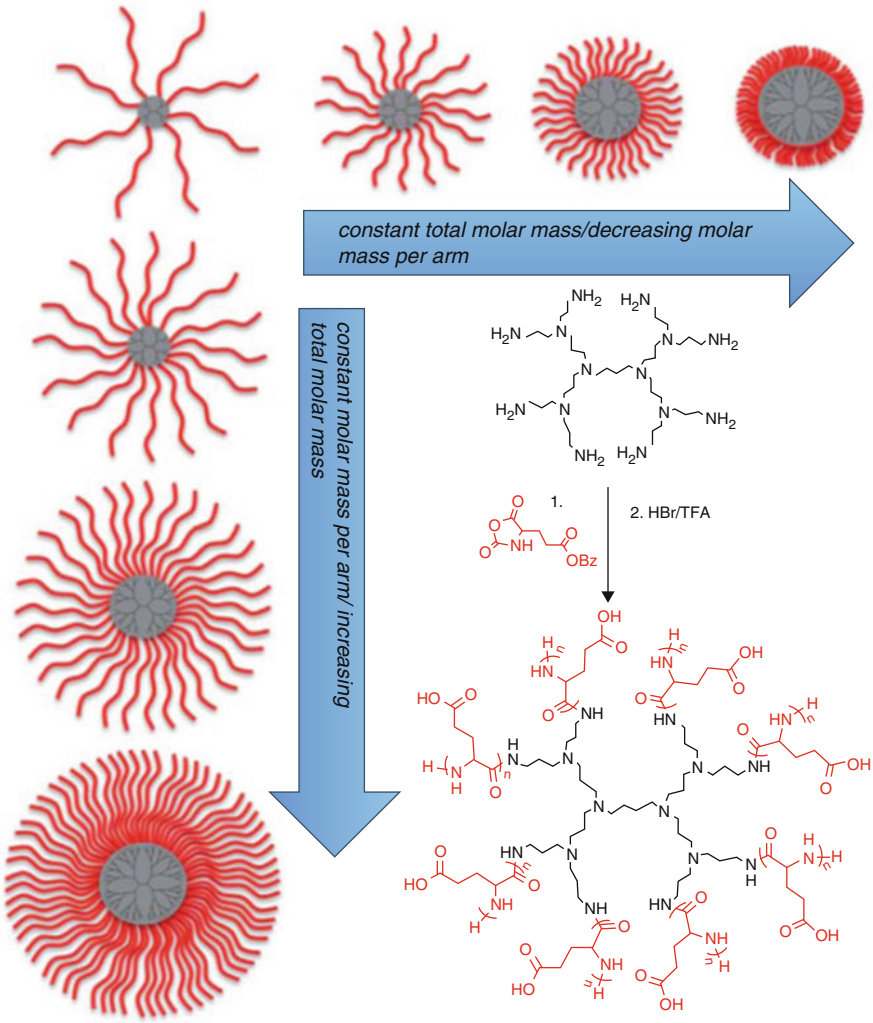

Fig. 17 Star polypeptides prepared from polypropylene imine dendrimers as initiators (Reproduced from Byrne et al. [42] with permission of The Royal Society of Chemistry)

using HDMS as initiator to prepare the arms, which was followed by the addition of L-cystine to afford core cross-linked stars (Fig. 18). The optimum cystine NCA/arm ratio for star formation was found to be highly dependent on the arm molecular weight. To obtain good stars, the ratio of cystine NCA to arms had to be controlled since higher ratios led to star–star coupling and gelation. The unreacted NCA groups in the core after the star formation were reacted with various amines bearing different functionalities for core functionalization. Deprotection of the arms afforded water-soluble core cross-linked stars, while reduction of the disulfide bonds with dithiothreitol (DTT) caused degradation of the stars to afford poly

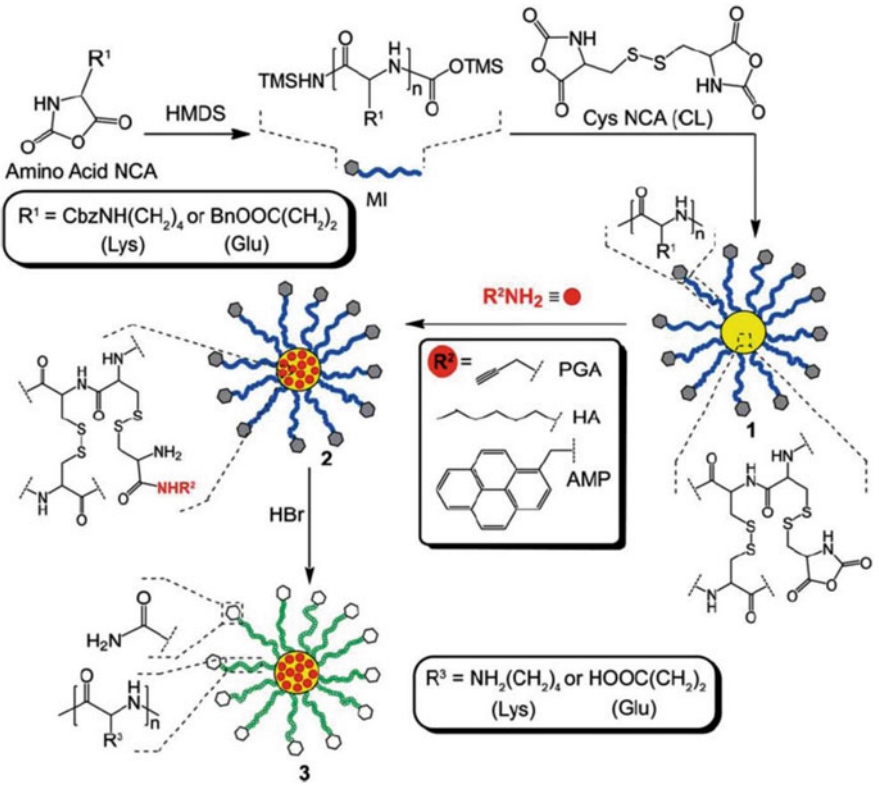

Fig. 18 Synthesis of core cross-linked polypeptide stars (Reproduced from Sulistio et al. [43] with permission of The Royal Society of Chemistry)

(ε-benzyloxycarbonyl-L-lysine-*b*-L-cysteine), which after exposure to oxygen formed organogels due to the random reformation of disulfide bonds.

4 Polypeptide-Based Hybrid Materials with Various Molecular Architectures

Polypeptides have been prepared in combination with various other polymers with either simple block structure or more complex molecular architectures to give rise to a number of new hybrid materials. The combination of highly self-organized polypeptide chains with other polymer chains with unique intrinsic properties led to a development of new materials with unprecedented properties that show a great potential in biomedical applications as well as other fields.

Block copolymer hybrid materials are usually prepared by using the first block with an amino end group as a macroinitiator for ROP of NCAs. An amine-

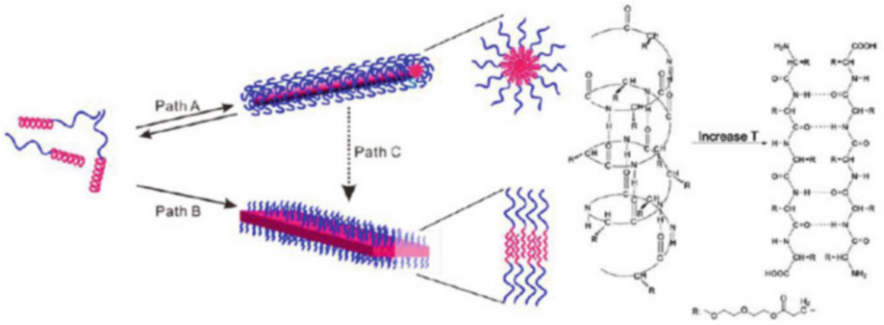

Fig. 19 Schematic representation of thermally induced self-assembly of PEO-*b*-poly(diethylene glycol-L-glutamate) copolymers (Reprinted with permission from Shen et al. [45]. Copyright 2013 American Chemical Society)

terminated PEO was used as a macroinitiator to prepare diblock PEO-poly(-β-benzyl-L-aspartate) copolymers, which were then coupled through amide bond formation, with *N*,*N*′-carbonyldiimidazole as coupling reagent, to eight pending carboxyl groups of the polyhedral oligomeric silsesquioxanes as a core to give star-shaped amphiphilic block copolymers, which self-assembled into micelles in aqueous medium [44]. Low dispersities ($Đ_M < 1.16$) were reported for the block copolymer; however, dispersities increased to 1.35–1.38 after the coupling to the core, indicating that coupling was not 100 % efficient. Micelles showed some potential as carriers for anticancer drug delivery.

An amine-terminated PEO was also used as a macroinitiator to prepare diblock copolymers with diethylene glycol-L-glutamate [45]. The second block shows thermoresponsive properties and adopts primarily helical conformation. The diblock copolymer upon increasing the temperature forms wormlike micelles, in which the poly(diethylene glycol-L-glutamate) formed the micelle core and maintained helical conformation. After prolonged thermal annealing, the helical conformation transforms to a β-sheet, which causes a rearrangement from wormlike micelles to nanoribbons, which is an irreversible process (Fig. 19).

Diethylene glycol-L-glutamate was used for preparation of star polypeptides as well [46]. Tetra(amine) compound with a disulfide bond was used as initiator core. The star polypeptides could self-assemble into micelles as well as hydrogels, which were reduction- and thermosensitive. The addition of DTT leads to a decrease of the size of the micelles due to the reduction of the disulfide bonds creating linear polypeptide chains with half the molecular weight instead of stars. The micelles exhibited LCST of about 40 °C which at higher temperatures leads to shrinking of the micelles as well (Fig. 20). Such a material shows great potential for triggered drug-release properties useful for on-demand drug delivery.

Chang et al. [47] prepared linear and comb-like terpolymers using amino-end-group functional PEO as macroinitiator for ROP to first prepare block copolymers with either poly(L-phenylalanine) or poly(L-serine) prepared from *O*-(*tert*-butyl)-L-serine NCA. Chain extension by polymerization of D,L-lactide in melt using Sn

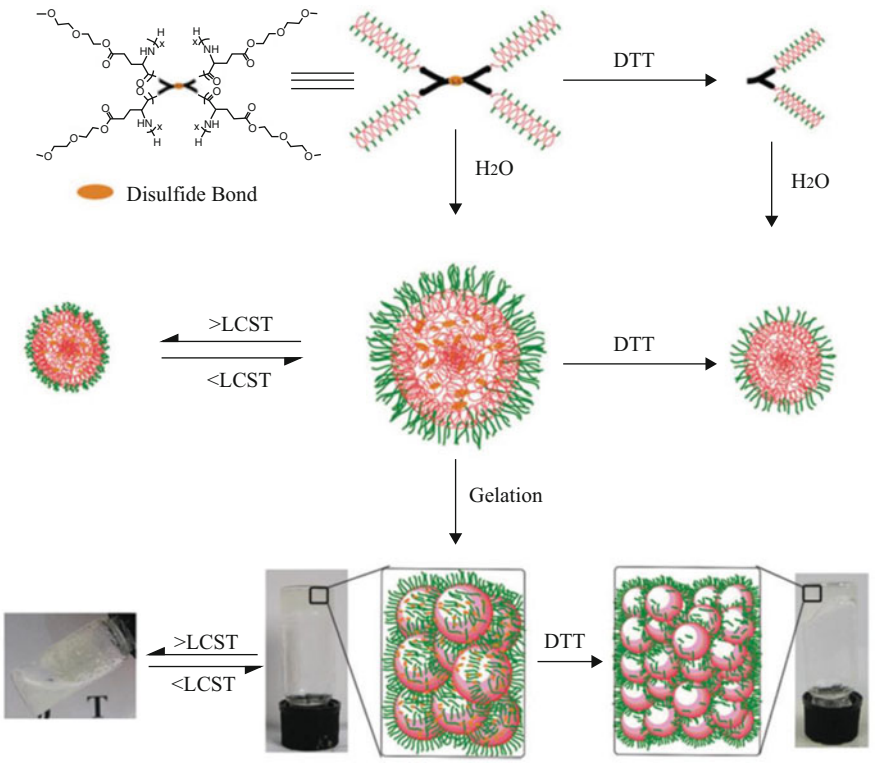

Fig. 20 Illustration of dual stimuli-sensitive micellization and hydrogelation process of diethylene glycol-L-glutamate-based star polypeptide (Reproduced from Liu et al. [46] with permission of The Royal Society of Chemistry)

$(Oct)_2$ afforded a linear terpolymer from block copolymer with poly (L-phenylalanine) and comb-like terpolymers when block copolymer with poly (L-serine) was used due to the hydroxyl group on each repeating unit of the poly (L-serine). Molecular weights were only determined from NMR, so no information on the dispersity was given. They have also prepared nanoparticles of the terpolymers by an emulsion-solvent evaporation method (Fig. 21).

Cheng et al. [48] prepared a series of PEO-cationic helical polypeptide copolymers with architectures ranging from diblock and triblock to 8-star and graft copolymers. Polymerization of γ-4-((2-(piperidin-1-yl)ethyl)aminomethyl)benzyl-L-glutamate was done using HDMS as initiator, while copolymers were obtained using mono-, di-, or 8-arm star PEO as initiator (Fig. 22), affording polymers with expected molecular weight and $Đ_M$ in range of 1.2, except for the star copolymer where obtained dispersity was a little higher. Polymers were tested for gene delivery, and diblock and triblock copolymers exhibited lower membrane activity and cytotoxicity and uncompromised gene transfection efficiencies compared to the

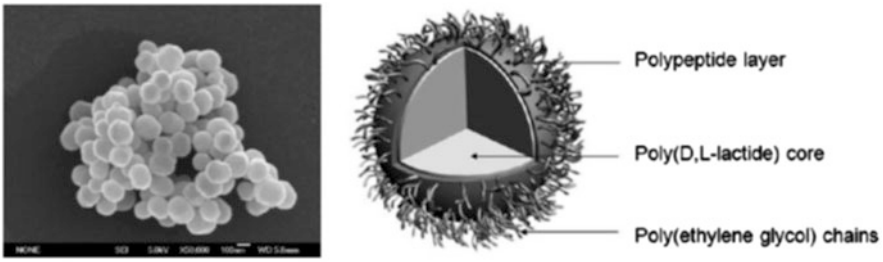

Fig. 21 Schematic representation of the PEO/polypeptide/poly(D,L-lactide) nanoparticle (*right*) and FE-SEM image of freeze-dried nanoparticles (*left*) (Reprinted with permission from Lee et al. [47]. Copyright © 2011 Wiley Periodicals, Inc.)

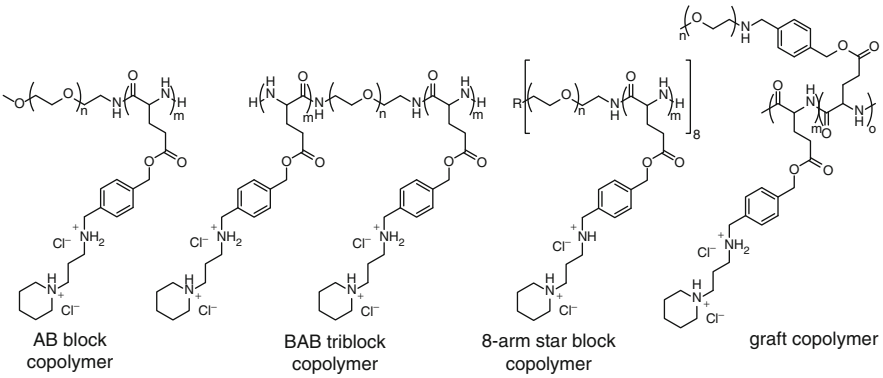

Fig. 22 Copolymers of PEO and cationic helical polypeptide poly(γ-4-((2-(piperidin-1-yl)ethyl) aminomethyl)benzyl-L-glutamate) with various macromolecular architectures

non-PEGylated homopolymer. Star copolymer displayed the highest membrane activity yet relatively low cytotoxicity.

After the polymerization of the first polymer block for hybrid materials, it is often necessary to prepare the macroinitiator by end-group transformation or deprotection reactions to afford the necessary amino functionality for ROP of NCAs. Diblock copolymers of highly branched polyethylene and γ-benzyl-L-glutamate were successfully prepared in this way. First, a highly branched polyethylene macroinitiator was synthesized by living polymerization of ethylene using an amine–imine nickel catalyst and a $ZnEt_2$ transfer agent. Hydroxyl end group was then transformed into amino group by end-capping reaction with Boc-protected valine. Such macroinitiator was then used for ROP of NCA (Fig. 23). $Đ_M$ of polyethylene macroinitiator was below 1.04; however, they were unable to determine the molecular weight characteristics of the diblock copolymer by size-exclusion chromatography. The diblock copolymer can form thermoreversible gels by self-assembled nanoribbons in toluene [49].

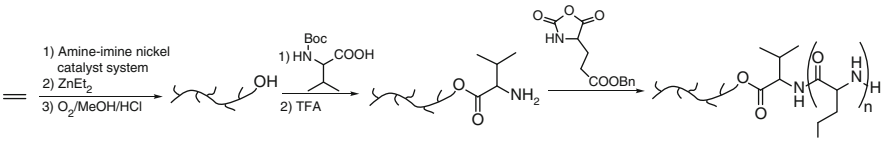

Fig. 23 Synthesis of diblock copolymer of highly branched polyethylene and γ-benzyl-L-glutamate

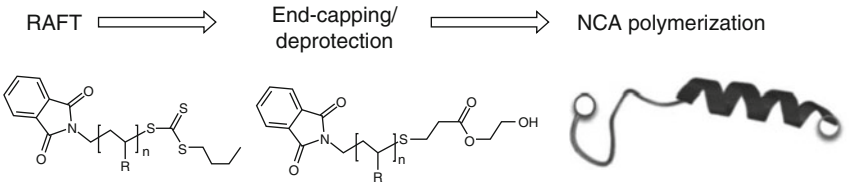

Fig. 24 Schematic representation of polypeptide hybrid materials synthesis using bifunctional RAFT agent (Reprinted with permission from Jacobs et al. [50]. Copyright © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim)

Polypeptide hybrid materials were prepared with poly(*n*-butyl acrylate), polystyrene, and poly(*N*-isopropyl acrylamide) as well, which were prepared by reversible addition–fragmentation chain transfer (RAFT) polymerization using bifunctional RAFT chain transfer agent with a phthalimide-protected amino group to afford well-defined polymers ($Đ_M < 1.1$) [50]. The trithiocarbonate group was first cleaved of with aminoethanol to form a thiolactone in the case of poly(*n*-butyl acrylate) or by simultaneous aminolysis/thiol–ene reaction with hydroxyethylacrylate for polystyrene and poly(*N*-isopropyl acrylamide). Effective cleavage of the trithiocarbonate group was essential since it can react with the amino groups. After purification, the phthalimide group was deprotected to afford amino-end-group functional macroinitiators, which were used for polymerization of γ-benzyl-L-glutamate or ε-benzyloxycarbonyl-L-lysine NCAs (Fig. 24). Block copolymers with expected molecular weight and low $Đ_M$ (<1.1) were obtained with poly(*n*-butyl acrylate) and polystyrene; however, in the case of poly(*N*-isopropyl acrylamide), a low molecular weight shoulder was observed indicating the presence of some starting material.

McCormick et al. [51] on the other hand used monofunctional RAFT chain transfer agent to polymerize the *N*-(2-hydroxypropyl)methacrylamide and transformed the trithiocarbonate end group by simultaneous aminolysis and thiol−ene Michael addition with *N*-(3-aminopropyl)methacrylamide to afford an amino-end-group functional macroinitiator. A chain extension was accomplished via ROP of γ-benzyl-L-glutamate NCA to afford block copolymers with different lengths and low dispersities (<1.2) (Fig. 25). The poly(L-glutamic acid) block shows a pH-responsive coil-to-helix transitions.

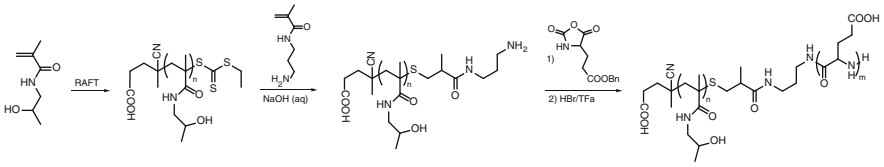

Fig. 25 Synthesis of poly(*N*-(2-hydroxypropyl)methacrylamide)-*b*-poly(L-glutamic acid)

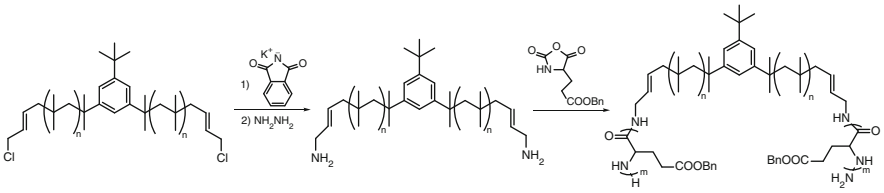

Fig. 26 Synthesis of poly(γ-benzyl-L-glutamate)-*b*-polyisobutylene-*b*-poly(γ-benzyl-L-glutamate) triblock copolymers

Faust et al. [52] prepared ABA triblock copolymers by polymerization of γ-benzyl-L-glutamate NCA from α,ω-primary amino-functional polyisobutylene. Polyisobutylene was prepared by cationic polymerization from a difunctional initiator, and the living cations were transformed to α,ω-chloroallyl polyisobutylene by reaction with 1,3-butadiene in hexanes/methylchloride. Nucleophilic substitution using phthalimide afforded after deprotection the α,ω-amino polyisobutylene, which was used for ammonium-mediated polymerization of γ-benzyl-L-glutamate NCA to obtain well-defined ABA copolymer with low $Đ_M$ (1.12) and molecular weights of about 5 kDa (Fig. 26). They have also performed a chain-extension experiment with 4,4′-methylene-bis(phenyldiisocyanate) to obtain $(ABA)_n$ multiblock copolymers; however, while they did obtain higher molecular weights, the $Đ_M$ increased to 2.80 with significant amount of what appeared to be unreacted ABA triblock copolymer as seen from SEC chromatogram.

Polypeptide-based amphiphilic graft terpolymers containing both hydrophilic PEO and hydrophobic poly(γ-benzyl-L-glutamate) attached to the acrylate backbone were synthesized by Huang et al. [53]. The pegylated backbone was first prepared by single-electron transfer-living radical polymerization (SET-LRP) of pegylated acrylic acid. The graft copolymer was then treated with lithium diisopropylamide followed by 2-bromoisobutyryl bromide to introduce bromide functional groups to the backbone, which were first transformed into azide groups by a nucleophilic substitution and then reduced to afford an amine-functionalized macroinitiator. The macroinitiator, with about 30 % of amino groups per repeating units, was then used for grafting from polymerization of γ-benzyl-L-glutamate NCA (Fig. 27). The macroinitiator had a very narrow molecular weight distribution ($Đ_M = 1.03$), which increased after grafting to $Đ_M < 1.21$ for the terpolymer. Amphiphilic graft terpolymers self-assembled into various micellar, which

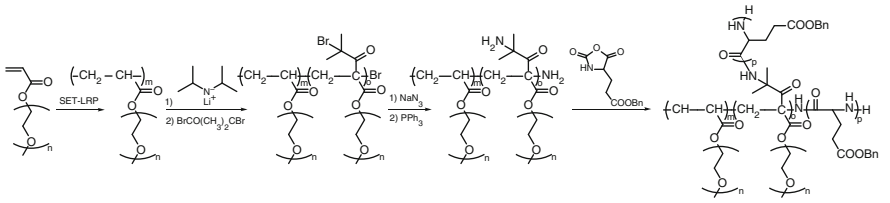

Fig. 27 Polypeptide-based amphiphilic graft copolymers prepared by successive SET-LRP of pegylated acrylic acid and ROP of γ-benzyl-L-glutamate NCA

morphologies depended on the initial water content, composition of the organic cosolvent, and length of the poly(γ-benzyl-L-glutamate) side chains.

Karatzas et al. [54] used unique features of anionic polymerization and the possibilities offered for the synthesis of end- or in-chain amino-functionalized polymers as well as of the living nature of the ROP of NCAs under high-vacuum conditions to prepare a variety of well-defined miktoarm star hybrids – macromolecular chimeras. The following structures were prepared: (PS)(PI)(PBLG or PBLL) (3 μ-stars), $(PS)_2$[P(α-MeS)](PBLG or PBLL), and $(PS)_2$(PBLG or PBLL)$_2$ (4 μ-stars), where PS is polystyrene, PI is polyisoprene, P(α-MeS) is poly (α-methylstyrene), PBLG is poly(γ-benzyl-L-glutamate), and PBLL is poly(ε-*tert*-butyloxycarbonyl-L-lysine) (Fig. 28). Diphenylethylene-functionalized polymers were first prepared and subsequently activated by reaction with a living polymer chain or *s*-BuLi followed by reaction with 1-(3-bromopropyl)-2,2,5,5-tetramethyl-aza-2,5-disilacyclopentane. The silyl-protected group was cleaved under acidic conditions to afford amine-functionalized macroinitiators for the polymerization of BLG and BLL NCAs to prepare the desired miktoarm stars (Fig. 28). Detailed characterization revealed the efficiency of this synthetic scheme and the homogeneity of prepared products; however, the miktoarm stars prepared from $(PS)_2(NH_2)_2$ showed broader molecular weight distributions, while $(PS)_4(NH_2)_2$ afforded multimodal distributions due to the significant steric hindrance of the in-chain functionalized macroinitiators.

(PS)(PI)(PBLL) miktoarm star terpolymer with two coil-like arms and an α-helical mesogenic polypeptide arm self-assemble in the solid state to form a hierarchical smectic self-assembly where the α-helical rod-like PBLL blocks stack to form pure polypeptide lamellae, while the PS and PI blocks form lamellae with an inner structure composed of rectangular cylinders (Fig. 29). This morphology is observed locally already after quick drop casting from chloroform solution, indicating the powerful driving force of the α-helical stacking in the formation of the structure; however, overall order is increased notably after thermal annealing even though partial thermal deprotection of PBLL block was observed [55].

ROP of NCA was employed to modify various nanoparticles and other surfaces with polypeptide chains as well. Materials prepared in such a way show a lot of promise for applications in the biomedical, electronic, or environmental fields.

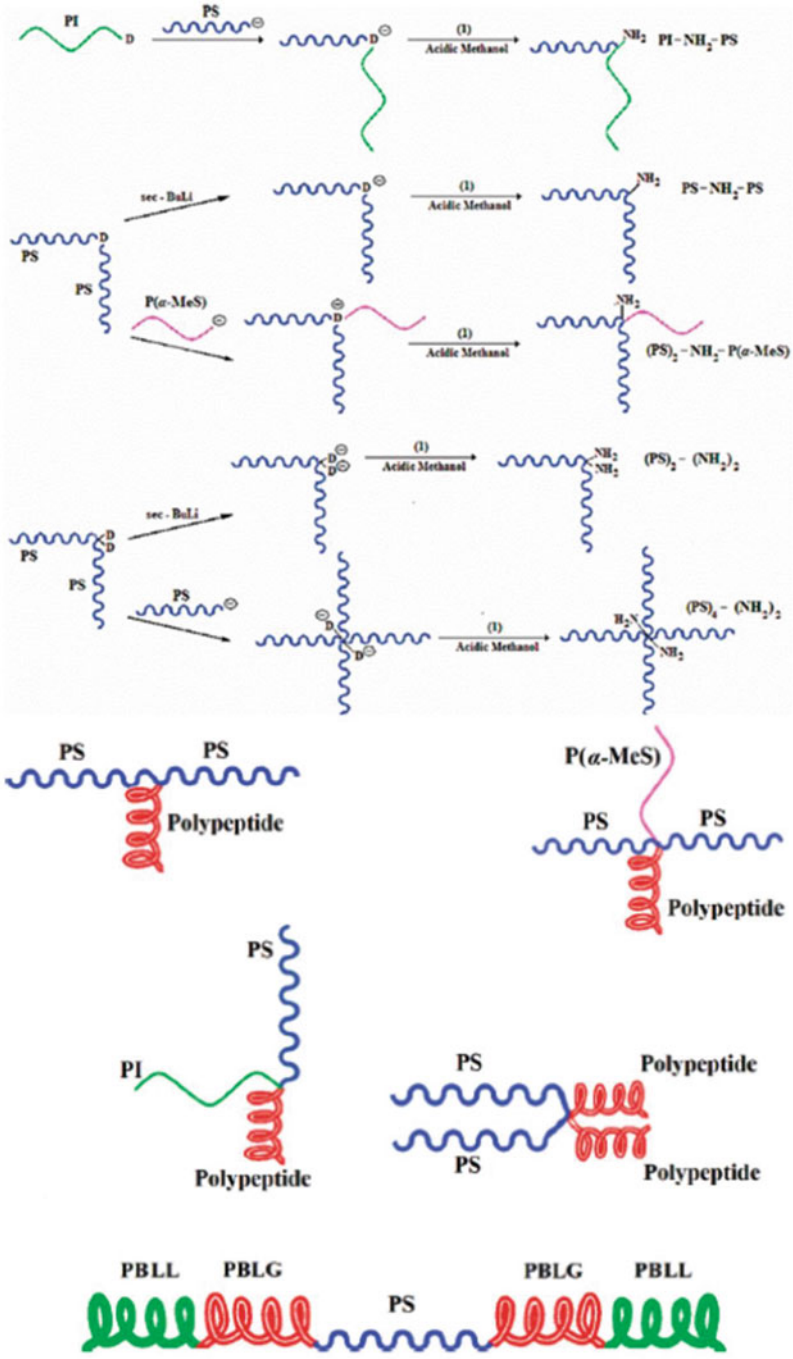

Fig. 28 Macromolecular chimeras based on polypeptides (Reprinted with permission from Karatzas et al. [54]. Copyright 2008 American Chemical Society)

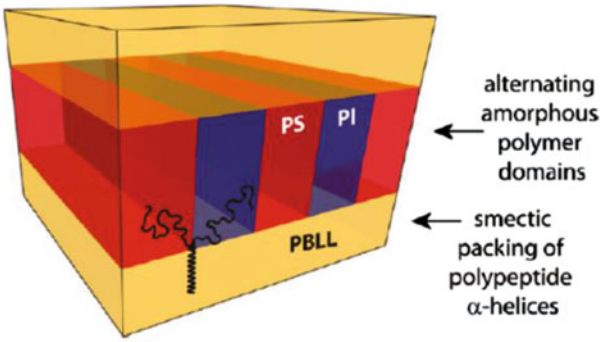

Fig. 29 Schematic illustration of the self-assembly of the (PS)(PI)(PBLL) miktoarm star terpolymer (Reprinted with permission from Junnila et al. [55]. Copyright 2010 American Chemical Society)

Chitin nanofibers have been successfully used for surface-initiated graft polymerization of γ-benzyl-L-glutamate NCA [56]. Chitin nanofibers were first partly deacetylated under very strong basic conditions to create amino groups, which were used as initiating sites. Grafted copolymer was then deprotected using milder basic conditions to afford carboxylic groups on the side chains. Carboxylic groups were then reacted with either remaining amino groups on the chitin backbone or with the amino group at the end of grafted polypeptides to produce a cross-linked biocompatible network film, which showed improved mechanical properties compared to the pure chitin nanofiber film.

Qiao et al. have prepared biodegradable hollow poly(L-glutamic acid) capsules by using silica nanoparticles as templates [57]. First hyperbranched poly(ethylene imine) macroinitiators were deposited on silica which enabled a highly dense polypeptide brush films to be formed after the γ-benzyl-L-glutamate NCA polymerization. High density of the growing chains leads to the cross-chain termination reactions between the propagating amine end groups of the growing polymer brushes and the side-chain benzyl ester protecting groups from neighboring polymer chains, to form stable cross-linked polypeptide films. Benzyl groups were then deprotected by acid hydrolysis, and the silica template was dissolved by HF/NH_4F solution to afford stable and dispersible hollow poly(L-glutamic acid) capsules (Fig. 30), which were successfully degraded using an endopeptidase papain.

Heise et al. [58] have prepared macroporous monoliths by polymerization of a high internal phase emulsion (HIPE) of a mixture of styrene, divinylbenzene, and 4-vinylbenzylphthalimide. After removal of the phthalimide, the free amino groups were used as initiators for ROP of γ-benzyl-L-glutamate or ε-*tert*-butyloxycarbonyl-L-lysine NCA to afford dense homogeneous coating of polypeptides throughout the internal polyHIPE surfaces (Fig. 31). Both types of polypeptide-grafted monoliths responded to pH by changes in their hydrophilicity, and successful bioconjugation with proteins was achieved, thus showing potential for use in biosensor as well as in bioseparation applications.

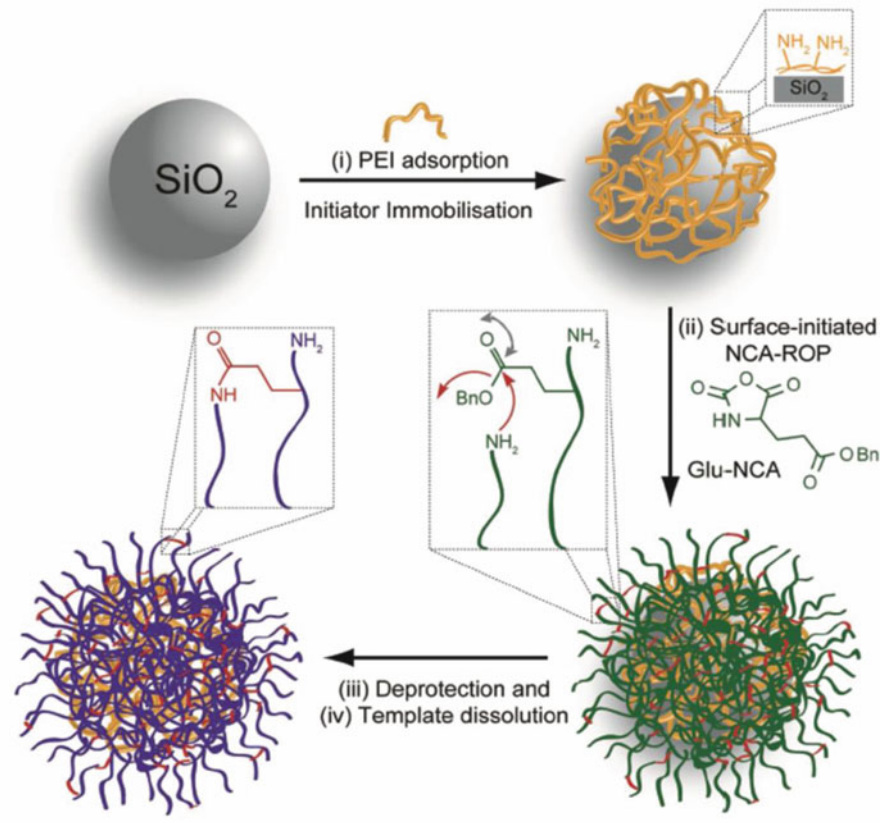

Fig. 30 Preparation of stable poly(L-glutamic acid) capsules using silica nanoparticles as template (Reprinted with permission from Harris et al. [57]. Copyright © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim)

Sakellariou et al. [59] grafted poly(L-proline) from an amine-modified surface of single-wall carbon nanotubes (SWNT) using high-vacuum technique. First, 1-benzocyclobutene-1′-phenylethylene was attached to the surface of carbon nanotubes by Diels–Alder cycloaddition. After reaction with *s*-butyl lithium, a carbanion was formed that reacted with 1-(3-bromopropyl)-2,2,5,5-tetramethyl-aza-2,5-disilacyclopentane which after the deprotection afforded an amine-functionalized carbon nanotubes with rather uniform polymer layer (Fig. 32). While grafted SWNTs are very well dispersed in $CHCl_3$, a small amount of trifluoroacetic acid causes aggregates to form due to the change in poly(L-proline) conformation from form I to form II, which is less soluble in organic solvents.

Brougham et al. [60] used surface-modified Fe_3O_4 nanoparticles bearing primary amino groups as initiators for ROP of γ-propargyl-L-glutamate to prepare

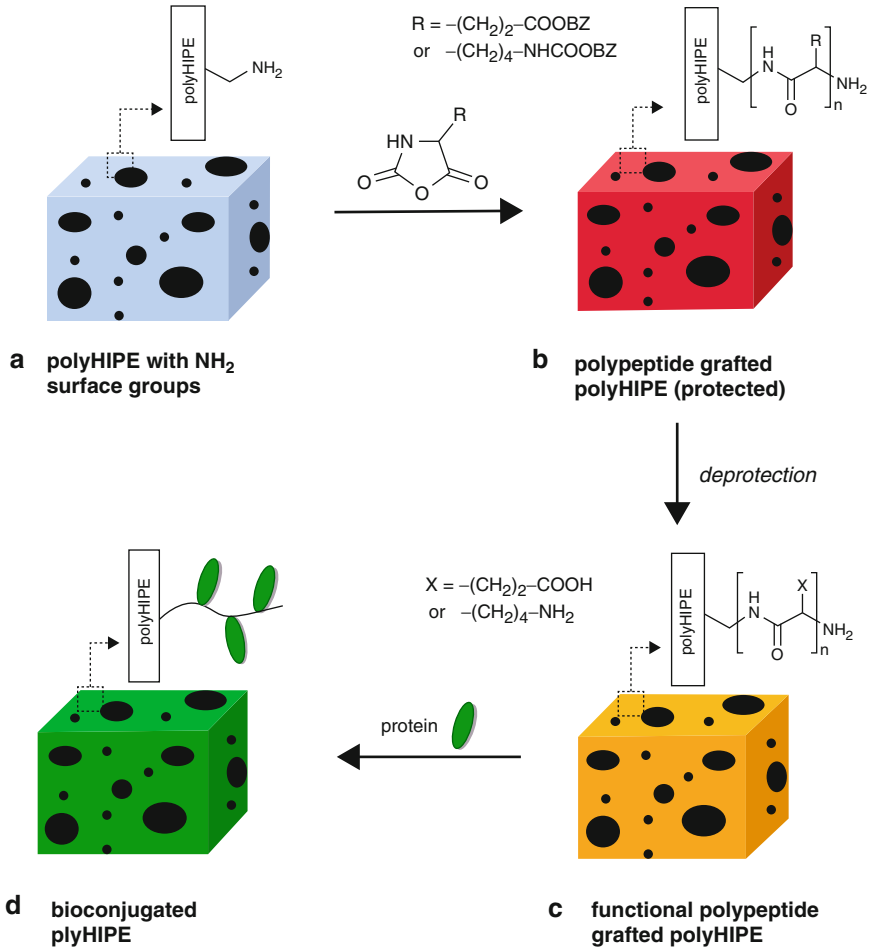

Fig. 31 (**a**) polyHIPE with NH2 surface groups (**b**) polypeptide grafted polyHIPE (protected). (**c**) Functional polypeptide grafted polyHIPE. (**d**) Bioconjugated polyHIPE

polypeptide-grafted magnetic nanoparticles. Polypeptide chains formed α-helix, and the pendant alkyne groups were reacted with azide-functionalized carbohydrate by copper-catalyzed cycloaddition reaction to afford biocompatible glycopeptide-grafted magnetic nanoparticles (Fig. 33). The grafted nanoparticles showed very low distribution of sizes suggesting that the stable suspensions are composed of fully dispersed grafted magnetic nanoparticles, which is critical for biological applications and were suitable for T_1-weighted magnetic resonance imaging as contrast agents.

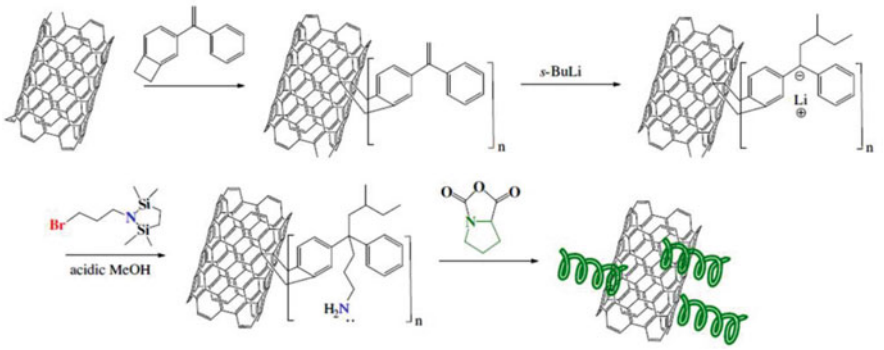

Fig. 32 Synthesis of the carbon nanotubes grafted with poly(L-prolin) (Reprinted from Gkikas et al. [59]. Copyright (2013), with permission from Elsevier)

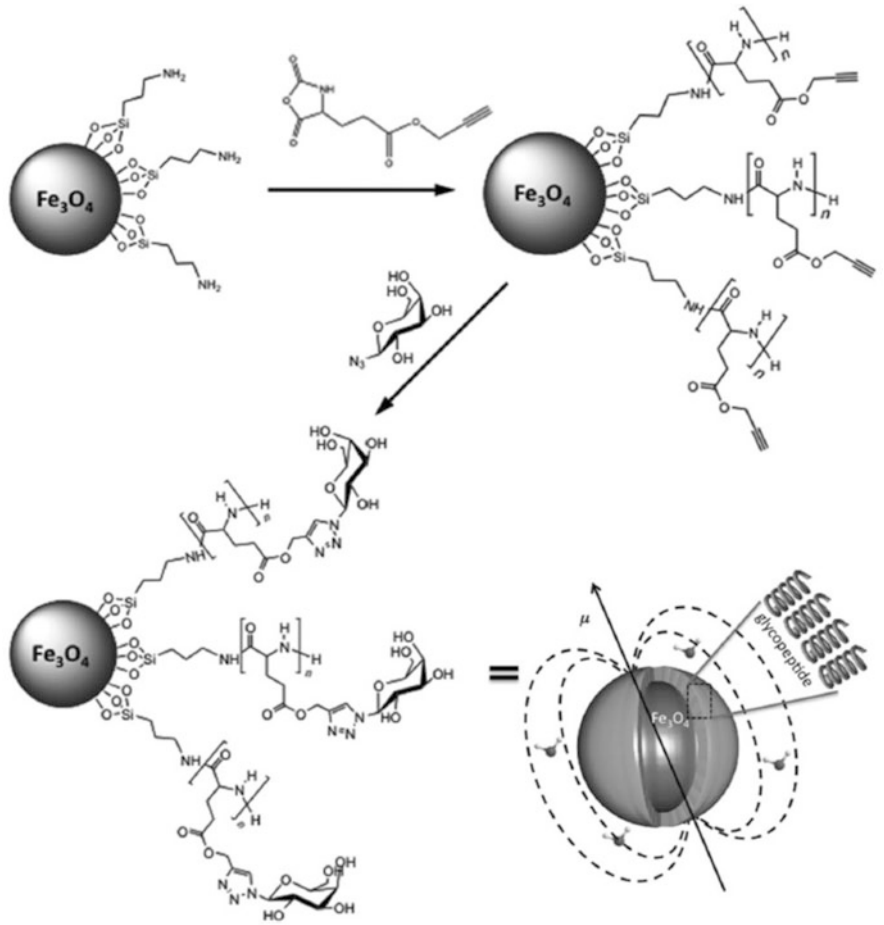

Fig. 33 Synthesis of glycopeptide-grafted magnetic nanoparticles (Reprinted with permission from Borase et al. [60]. Copyright © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim)

Abbreviations

AMM	Activated monomer mechanism
Bipy	2,2′-bipyridyl
COD	1,5-cyclooctadiene
$Đ_M$	Dispersity
DTT	Dithiothreitol
HMDS	Hexamethyldisilazane
HIPE	High internal phase emulsion
LCST	Lower critical solution temperature
NAM	Normal amine mechanism
NCA	*N*-carboxyanhydrides
NHC	*N*-heterocyclic persistent carbenes
P(α-MeS)	Poly(α-methylstyrene)
PAla	Polyalanine
PBLG	Poly(γ-benzyl-L-glutamate)
PBLL	Poly(ε-tert-butyloxycarbonyl-L-lysine)
pDNA	Plasmid deoxyribonucleic acid
PEO	Poly(ethylene oxide)
PI	Polyisoprene
PLEU	Poly(L-leucine)
PLGA	Poly(L-glutamic acid)
PLL	Poly(L-lysine)
POBT	Poly(*O*-benzyl-L-tyrosine)
PS	Polystyrene
PZLL	Poly(ε-benzyloxycarbonyl-L-lysine)
RAFT	Reversible addition–fragmentation chain transfer
ROP	Ring-opening polymerization
SET-LRP	Single-electron transfer-living radical polymerization
siRNA	Small interfering ribonucleic acid
SWNT	Single-wall carbon nanotubes
TMS	Trimethylsilyl

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