Journal of Controlled Release 193 (2014) 9–26

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Role of non-covalent and covalent interactions in cargo loading capacity and stability of polymeric micelles

Xiyu Ke [a,b,1], Victor Wee Lin Ng [a,1], Robert J. Ono [c], Julian M.W. Chan [c], Sangeetha Krishnamurthy [a,b], Ying Wang [d], James L. Hedrick [c], Yi Yan Yang [a,*]

[a] Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, Singapore 138669, Singapore
[b] NUS Graduate School for Integrative Sciences & Engineering (NGS), National University of Singapore, 28 Medical Drive, Singapore 117456, Singapore
[c] IBM Almaden Research Center, 650 Harry Road, San Jose, CA 95120, USA
[d] Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore

ARTICLE INFO

Article history:
Received 7 April 2014
Accepted 24 June 2014
Available online 15 July 2014

Keywords:
Polymeric micelles
Hydrophobic interaction
Hydrogen bonding interaction
Ionic interaction
Chemical cross-linking
Chemical conjugation

ABSTRACT

Polymeric micelles self-assembled from biodegradable amphiphilic block copolymers have been proven to be effective drug delivery carriers that reduce the toxicity and enhance the therapeutic efficacy of free drugs. Several reviews have been reported in the literature to discuss the importance of size/size distribution, stability and drug loading capacity of polymeric micelles for successful *in vivo* drug delivery. This review is focused on non-covalent and covalent interactions that are employed to enhance cargo loading capacity and *in vivo* stability, and to achieve nanosize with narrow size distribution. In particular, this review analyzes various non-covalent and covalent interactions and chemistry applied to introduce these interactions to the micellar drug delivery systems, as well as the effects of these interactions on micelle stability, drug loading capacity and release kinetics. Moreover, the factors that influence these interactions and the future research directions of polymeric micelles are discussed.

1. Introduction

Amphiphilic block copolymers can spontaneously self-assemble into core/shell nanostructured micelles in aqueous solution at concentrations above the critical micelle concentration (CMC). Polymeric micelles are promising carriers for the delivery of therapeutics, and several types of polymeric micelles have already seen application in hospital settings or have been investigated in clinical trials [1–5]. Compared with other drug delivery systems, polymeric micelles have a number of important properties. These include a unique core/shell structure composed of a hydrophilic exterior and a hydrophobic interior, nanosize, and easy modification of core functionalities and surface chemistry. The core of micelles is formed from the hydrophobic moieties of copolymers, into which hydrophobic drug molecules can be loaded. Drug loading capacity is a key parameter of polymeric micelles, which is mainly affected by interactions between the drug and the micellar core. The hydrophilic shell protects the loaded drugs from enzymatic degradation and uptake by mononuclear phagocytes, macrophages and reticuloendothelial systems in the liver, spleen and bone marrow, hence prolonging the blood circulation time of the drug [4,6]. In addition, polymeric micelles having sizes of 20–200 nm are large enough to escape extravasation from normal vessel walls and avoid premature elimination *via* glomerular filtration in the kidneys, but small enough to permeate through leaky blood vessels and stay within the tumor tissues due to compromised lymphatic filtration, otherwise known as the enhanced permeation and retention (EPR) effect [7,8]. On the cellular level, the nanosize of polymeric micelles allows for easy cellular uptake, and offers an endocytosis internalization pathway, overcoming multidrug-resistance caused by drug efflux mechanisms [9]. The size and its distribution are also important considerations in polymeric micelles, determined primarily by polymer molecular weight, relative content of hydrophobic moiety, drug loading level, and micelle fabrication conditions. In addition to drug loading capacity and size, the stability of polymeric micelles is also essential for successful *in vivo* drug delivery, and is governed by thermodynamic and kinetic principles [10,11]. The thermodynamic stability of micelles is measured by the CMC of polymers. Polymers having lower CMCs form micelles with greater thermodynamic stability. The micelles would remain intact following systemic administration if the polymer concentration in the blood stream is above its CMC. CMC of micelles affects blood circulation. For example, 74% of micelles made from poly(ethylene glycol)-*b*-polycaprolactone with CMC of 38 mg/L was still found in mouse blood after 24 h of circulation as compared to 33% of micelles formed from Pluronic P85 (poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(-ethylene oxide)) with CMC of 300 mg/L [12,13]. On the other hand, the

* Corresponding authors. Tel.: +65 6824 7106; fax: +65 6478 9084.
E-mail address: yyyang@ibn.a-star.edu.sg (Y.Y. Yang).
[1] These authors contributed to this work equally.

http://dx.doi.org/10.1016/j.jconrel.2014.06.061
0168-3659/

kinetic stability of micelles determines how fast the micelles dissociate into individual polymer chains. The micelles with high kinetic stability can still be stable when circulating in the blood stream even at polymer concentrations in the proximity of its CMC [12]. The kinetic stability is mainly dominated by the interactions between the core and the loaded drug, and between the hydrophobic moieties, as well as the proportion of the hydrophobic to the hydrophilic moieties. Another important factor that influences the kinetic stability of micelles is glass transition temperature T_g. The drug release of micelles self-assembled from polymers containing a hydrophobic chain having T_g higher than 37 °C is low under the simulated physiological condition due to slow diffusion. If the drug is molecularly dissolved within micellar core, it may act as a plasticizer and lower the T_g of the core-forming block, which may accelerate drug release. In contrast, if the drug is present as a crystal, it may act as a reinforcing filler, which may cause an increase in T_g. The effect of T_g on the kinetic stability of micelles has been reviewed in the literature [14], and it will not be discussed in detail in this review.

The installation of various functionalities in copolymers to enhance drug loading capacity and micelle stability can be achieved by using synthetic chemical methods such as controlled radical polymerization, ring-opening methathesis polymerization (ROMP), and organocatalytic ring-opening polymerization (ROP). These controlled polymerization techniques and their functional group tolerance allow for the tuning of the micellar core through non-covalent [15–22] and covalent [23,24] interactions, and achieve stimulus-sensitive drug release [23,25]. In addition, the surface chemistry of micelles can be designed to chemically or physically attach biological ligands that can recognize specific receptors over-expressed on certain cells for active targeting [26–30] and to prolong blood circulation time [31–35]. In this review, the role of non-covalent (*e.g.* hydrophobic, hydrogen bonding, and ionic) and covalent interactions together with the role of polymer architecture in stability, drug loading capacity, and drug release kinetics of polymeric micelles will be described through specific examples. The chemistry to incorporate these interactions into micellar drug delivery systems will be provided. The strategies for the development of ideal micellar drug delivery systems will be discussed.

2. Hydrophobic interaction

Hydrophobic interactions are the most extensively studied non-covalent interactions and the driving force for the spontaneous self-assembly of amphiphilic diblock copolymers into micelles in water. Hydrophobic interactions are widely employed in the design of most micellar drug delivery systems. Amphiphilic copolymers with various architectures, including block, graft, star and hyperbranched copolymers (Fig. 1A), have been investigated for micellar drug delivery, and structure–function relationships have been established as a function of architecture. Among these copolymers, the block copolymers are the most commonly reported materials used to prepare micelles for drug delivery. Genexol®-PM is the first commercially available polymeric micelle formulation for the treatment of non-small-cell lung carcinoma, ovarian cancer, breast cancer and gastric cancer [1]. In this formulation, the hydrophobic anticancer drug Paclitaxel (PTX) was loaded into monomethoxy poly(ethylene glycol)-*block*-poly(D,L-lactide) (mPEG–P_{DL}LA) micelles through hydrophobic interactions [36,37]. Kataoka et al. also developed PTX-loaded polymeric micelles (denoted as NK105) for PTX delivery [38]. In this case, poly(ethylene glycol)–poly(aspartic acid) (PEG–P(Asp)) block copolymer was conjugated with 4-phenyl-1-butanol through acid groups in the P(Asp) block to increase hydrophobicity for improved PTX loading. On colon 26-bearing CDF1 mice, the plasma and tumor area under the curve values of NK105 were about 90-fold and 25-fold higher than that of free PTX. On a human colorectal cancer cell line HT-29 xenograft, the antitumor activity of NK105 at a dose of 25 mg/kg was comparable to that of free PTX at a dose of 100 mg/kg. After treatment with NK105 at a dose of 100 mg/kg, tumors in all mice disappeared. Compared with free DOX, NK105 showed milder neurotoxicity, which was demonstrated by both histopathological ($p < 0.001$) and physiological ($p < 0.05$) methods [38]. A phase II clinical trial of NK105 against advanced or recurrent gastric cancer was successfully conducted, and the results were promising [39].

The hydrophobic interactions within micelles self-assembled from linear copolymers can be affected by the hydrophobicity of the core-forming

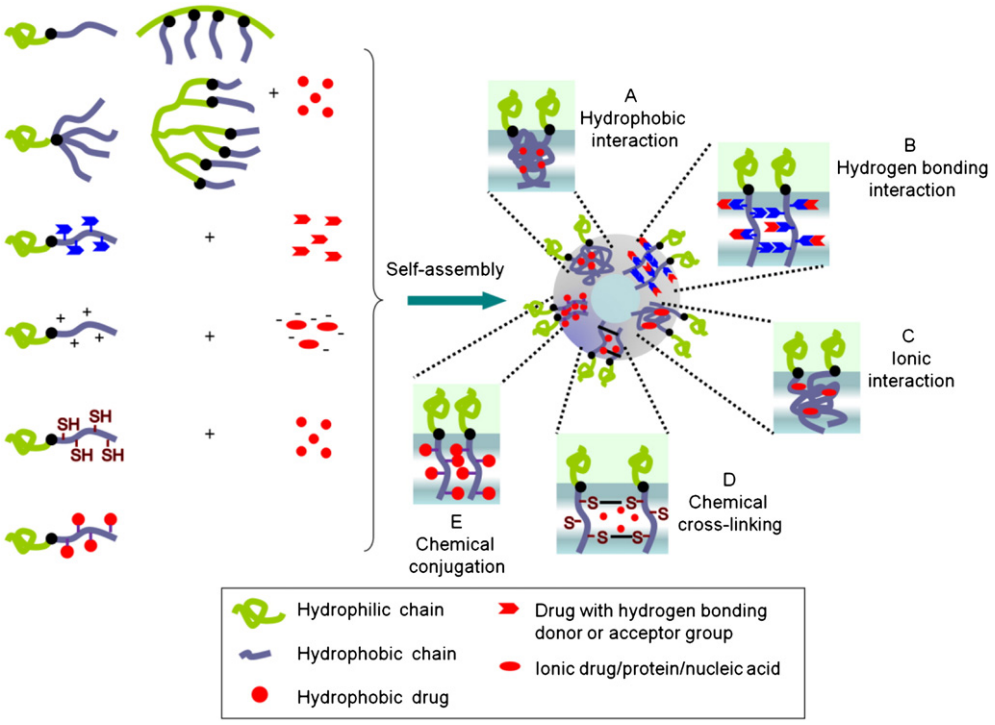

Fig. 1. Schematic presentation of micellar drug delivery systems self-assembled through (A) hydrophobic interaction; (B) hydrogen bonding interaction; (C) ionic interaction; (D) chemical cross-linking and (E) chemical conjugation.

hydrophobic moieties and the drugs, as well as the compatibility between the polymer and drug. Varshosaz et al. synthesized three amphiphilic block copolymers with different hydrophobic lengths by coupling mPEG with myristic acid, stearic acid and behenic acid *via* an ester linkage [40]. Increasing the length of the fatty acid chain reduced CMC and enhanced the interactions between the micellar core and the hydrophobic drug etoposide, leading to higher solubility and loading level of etoposide. The hydrophobicity of the encapsulated drug also affects hydrophobic interactions with the micellar core. Alexander et al. found that incorporating 0.6 wt.% flurbiprofen into Pluronic P123 and P103 micelles led to reduced CMC and promoted micellization [41]. It was also found that P123 micelles with a greater proportion of hydrophobic to hydrophilic block were more suitable than P103 for flurbiprofen loading due to the larger size of micellar core.

PTX has a rigid chemical structure with 11 stereocenters (4 R and 7 S) and 3 benzene rings, and tends to self-associate into long fibers [42]. Yang and coworkers took advantage of this self-associative property and demonstrated fiber-like supramolecular structures having hierarchical order (~200 nm radius and ~40–200 μm length) formed from the co-assembly of stereo-regular poly(ethylene glycol)–*block*–polylactide (PEG-*b*-PLA) block copolymers with PTX. Importantly, these PTX-loaded block copolymer complexes possessed a PEG shell and showed stable sustained release of the drug under the simulated physiological conditions (Fig. 2) [43]. In a complementary study, dissipative particle dynamic simulations were carried out on the pure PTX and the PTX-loaded micelles to elaborate the microstructure of the fibers and confirmed the experimental observations [44]. The introduction of aromatic end groups (*e.g.* benzoyl and naphthoyl) to the core-forming block of PEG-*block*-poly(caprolactone) enhanced the polymer–drug interactions and significantly improved PTX loading efficiency in the micelles [45]. Using an amphiphilic cholesterol-containing copolymer, poly{(N-methyldietheneamine sebacate)-*co*-[(cholesteryl oxocarbonylamido ethyl) methyl bis(ethylene) ammonium bromide] sebacate} (P(MDS-*co*-CES)), PTX was encapsulated into nanosized micelles with a high loading level of ~14 wt.% through a simple self-assembly procedure without homogenization or sonication [46–48]. In a recent study, diblock copolymers of mPEG and cholesterol-containing biodegradable polycarbonate ($mPEG_{113}$-*b*-P(MTC-$Chol_x$-*co*-TMC_y)$_{x+y}$) were synthesized and employed to fabricate micelles for the delivery of PTX (Fig. 3A) [16]. The polymer with the optimal composition (x = 11; y = 30) had the lowest CMC (1.5 mg/L) and the highest PTX loading capacity (15 wt.%), and formed the most compact PTX-loaded micelles with extremely small size (36 nm) and narrow size distribution (polydispersity index: 0.07). The PTX-loaded micelles exhibited excellent kinetic stability, and preferably accumulated in the tumor tissues *via* EPR effect after i.v. injection in a 4T1 mouse breast cancer model (Fig. 3B and C). The cholesterol group was introduced to the polymers because of its rigid hydrophobic structure that promotes self-association, which may improve the hydrophobic block–PTX compatibility. Moreover the self-associative or liquid crystalline character of the cholesterol-containing hydrophobic block is believed to be responsible for the high kinetic stability and small size of the PTX-loaded micelles with circulation times as long as five days.

Compared with linear diblock copolymers, graft copolymers were reported to have lower CMC and greater drug loading capacity. For example, Jiang et al. compared a diblock copolymer of methoxy poly(ethylene glycol)-*b*-poly(5-allyloxy-1,3-dioxan-2-one) (mPEG-*b*-PATMC) with a graft copolymer, which was formed by grafting PATMC onto mPEG-*b*-PATMC (mPEG-*b*-(PATMC-*g*-PATMC)) [49]. The graft copolymer formed micelles at a much lower concentration and the particle size of graft copolymer micelles was smaller than that of micelles formed from the diblock copolymer mPEG-*b*-PATMC. The graft copolymer micelles also had greater drug loading capacity and drug loading efficiency. In another study, reducible amphiphilic polyamide amine-*g*-poly(ethylene glycol) (PAA-*g*-PEG) graft copolymers containing disulfide linkages were synthesized and utilized to load doxorubicin (DOX) into micelles. The micelles prepared from the polymer with the optimal compositions yielded high drug loading capacity (25 wt.%) and nanosize (44 nm), suppressed tumor growth more effectively than free DOX in a 4T1 mouse breast cancer model [50]. Hedrick and coworkers have also reported systems based on graft amphiphilic block copolymers [15]. Aliphatic polycarbonate monomers having either an

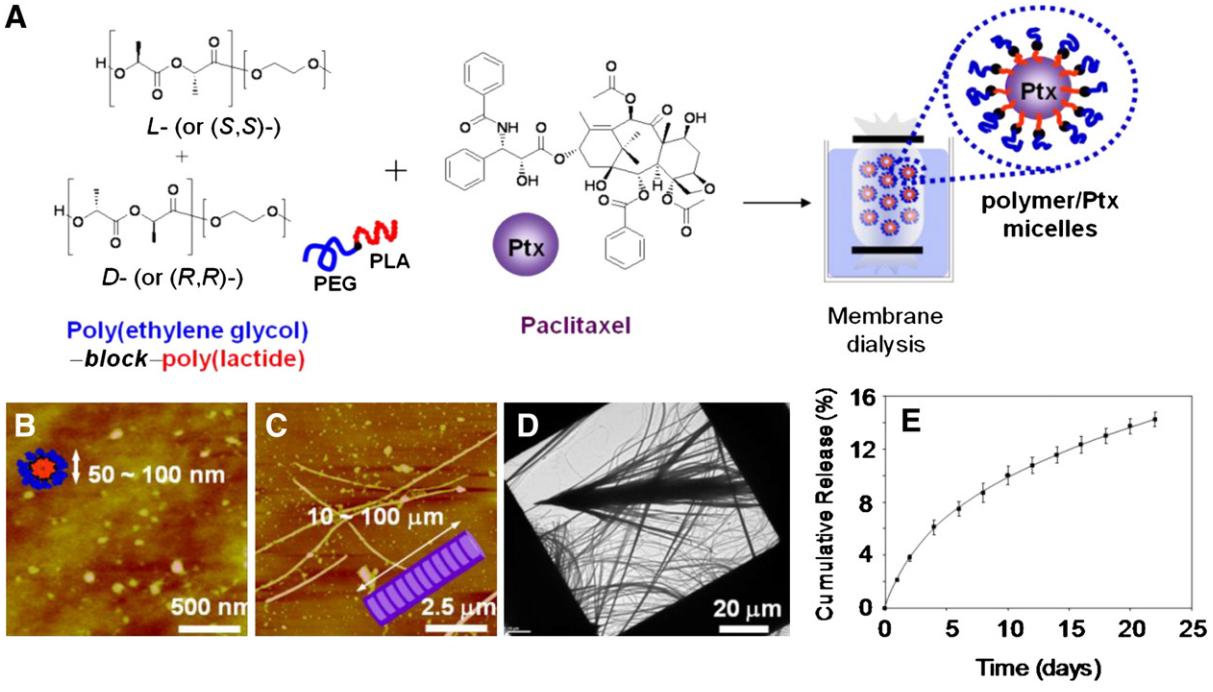

Fig. 2. Self-assembly of PEG-*b*-PLA and paclitaxel through stereocomplexation. (A) Schematic for preparation of PEG-*b*-PLA/paclitaxel supramolecular structures using membrane dialysis; AFM and transmission electron microscopy (TEM) images showing morphologies formed from PEG-*b*-PLLA + PEG-*b*-PDLA (B), and (PEG-*b*-PLLA + PEG-*b*-PDLA)/paclitaxel mixture (C, D). *In vitro* drug release profile of paclitaxel-loaded stereoblock copolymer assemblies (PEG-*b*-PLAs) (5k-*b*-10k) at pH 7.4 and 37.0 ± 0.1 °C (E).
Reprinted with permission from reference [43].

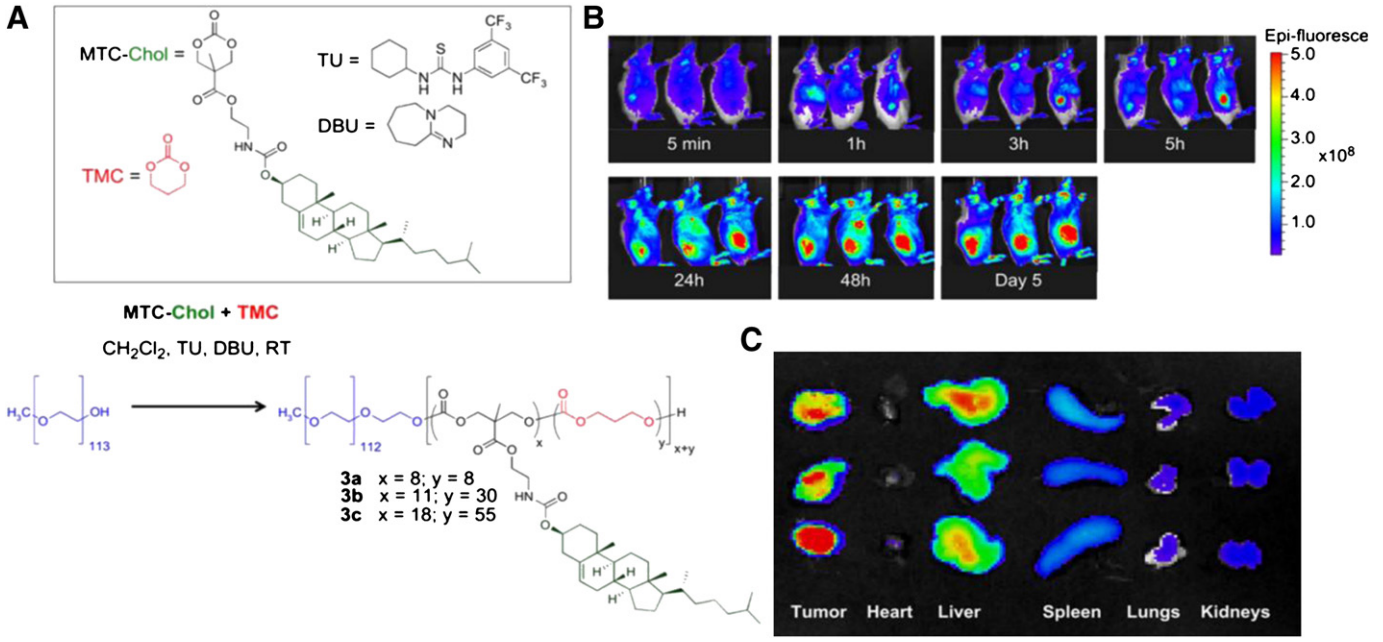

Fig. 3. PTX-loaded micelles from diblock copolymers of mPEG and cholesterol-containing biodegradable polycarbonate ($mPEG_{113}$-*b*-P(MTC-$Chol_x$-co-TMC_y)$_{x+y}$) for passive targeting of tumor. (A) Synthesis of block copolymers; (B) near-infrared fluorescence images of 4T1 tumor-bearing mice following intravenous administration of DiR-loaded mPEG113-*b*-P(MTC-$Chol_{11}$-*co*-TMC_{30}) nanoparticles. (C) Near-infrared fluorescence image of various organs at 5 days post intravenous administration. Reprinted with permission from reference [16].

ethyl ester or protected alcohol group (MTC-ethyl cyclic carbonate monomer and MTC-tetrahydro-2H-pyran-2-yloxy cyclic carbonate monomer, respectively) were randomly copolymerized using mPEG as a macromolecular initiator through metal-free organocatalytic living ring-opening polymerization (ROP) to give narrowly dispersed diblock copolymers. Deprotection of the tetrahydropyranyl ethers generated free hydroxyl groups in the hydrophobic polycarbonate block, which serve as initiation sites for the subsequent grafting of poly(lactide) brushes to the hydrophobic block, mPEG-*b*-PMTC(Et-*co*-HE). The graft polymers have well-defined molecular compositions, CMC of ~0.3 to 1.1 mg/L, nanosize (~26–34 nm) and narrow size distribution (polydispersity indeces: ~0.10–0.12). Grafting degree can be varied to modulate particle size and drug loading capacity. Du et al. synthesized stearate-*g*-dextran (Dex–SA) graft copolymers with different grafting degrees of hydrophobic SA. Increasing the grafting degree led to smaller particle size, slightly higher loading capacity and encapsulation efficiency for DOX [51]. These examples demonstrated that graft amphiphilic copolymers offer low CMC and high drug loading capacity. However, graft polymers in which hydrophobic chains are grafted onto a hydrophilic polymer backbone or hydrophilic chains grafted onto a hydrophobic polymer backbone may form a loosely packed micellar core [52], leading to low stability in the blood stream.

In addition to graft polymers, hyperbranched and star copolymers have also been extensively explored for micelle preparation to enhance drug loading capacity since the multiple hydrophobic chains may increase interactions with hydrophobic drugs. For instance, micelles prepared from hyperbranched polypeptide and poly(ethylene oxide) (PEO) block copolymers were used to encapsulate DOX [53]. The hyperbranched block copolymers were synthesized in two steps. Hyperbranched poly(ε-benzyloxycarbonyl-L-lysine) (HPlys) with multiple alkyne groups was synthesized *via* click chemistry by using Plys with α-thiol and ω-alkyne terminal groups, which was further conjugated with thiol-functionalized PEO to afford HPlys-*b*-PEO block copolymers. HPlys-*b*-PEO had a 5-fold lower CMC, higher DOX loading level/efficiency, greater yield and a more sustained drug release profile than its diblock analog Plys-*b*-PEO. Li et al. reported star-shaped block copolymers poly (ε-caprolactone)-*b*-poly(2-hydroxyethyl methacrylate) (sPCL-*b*-PHEMA) with three arms and six arms [54]. Star-shaped PCL polymers were first prepared by using 1,1,1-tris(hydroxymethyl) ethane and dipentaerythritol as multifunctional initiators through organometallic ROP of ε-CL. The end hydroxyl groups of sPCL were reacted with 2-bromoisobutyryl bromide to yield sPCL-Br as macroinitiator for further polymerization of 2-hydroxyethyl methacrylate (HEMA) through atom transfer radical polymerization (ATRP). A series of 3-arm and 6-arm sPCL-*b*-PHEMA polymers were synthesized, and employed to load PTX into micelles. Although PTX loading level was generally low (4.2–9.2 wt.%), the 6-arm sPCL-*b*-PHEMA micelles had higher loading capacity as compared to the 3-arm polymeric micelles. Since the proportion of hydrophobic to hydrophilic block in each arm of 3-arm and 6-arm polymers was similar, the CMC values for both polymers were the same. Hammond's group reported elegant studies on linear dendritic amphiphilic block polymers, where poly(benzyl-L-aspartate) (PBLA) is the linear hydrophobic block and a generation four biodegradable polyester dendron conjugated with sixteen short hydrophilic PEG chains is the hydrophilic block [55]. There is a carboxylic acid group on the end of each PEG chain, which was reacted with folate through DCC/NHS coupling chemistry. Folate targets folate receptors that overexpress on the surface of many types of cancer cells such as KB mouth epidermal carcinoma cells. The mixture of folate-functionalized dendron and non-functionalized dendron formed "patchy" micelles with folate present in cluster arrangements on the surface. The micelles with an optimal cluster arrangement of folate groups effectively targeted KB tumor in nude mice bearing KB xenografts after tail vein injection. In a subsequent study, PTX was loaded into the optimal "patchy" micelles having a diameter of 80 nm and a loading level of 2.5 wt.% (Fig. 4A). *In vitro* release study indicated that these micelles would be stable during circulation in the blood (at pH 7.4, only 15 ± 6% PTX released at 48 h), but readily released within the endosomes (at pH 5.5, 68 ± 10% PTX released at 48 h) (Fig. 4B). These micelles suppressed the tumor growth more effectively than the micelles without folate and free PTX, and increased survival (Fig. 4C) [56].

3. Hydrogen bonding interaction

As mentioned previously, there are a number of parameters that should be taken into consideration in the design of polymeric micelles,

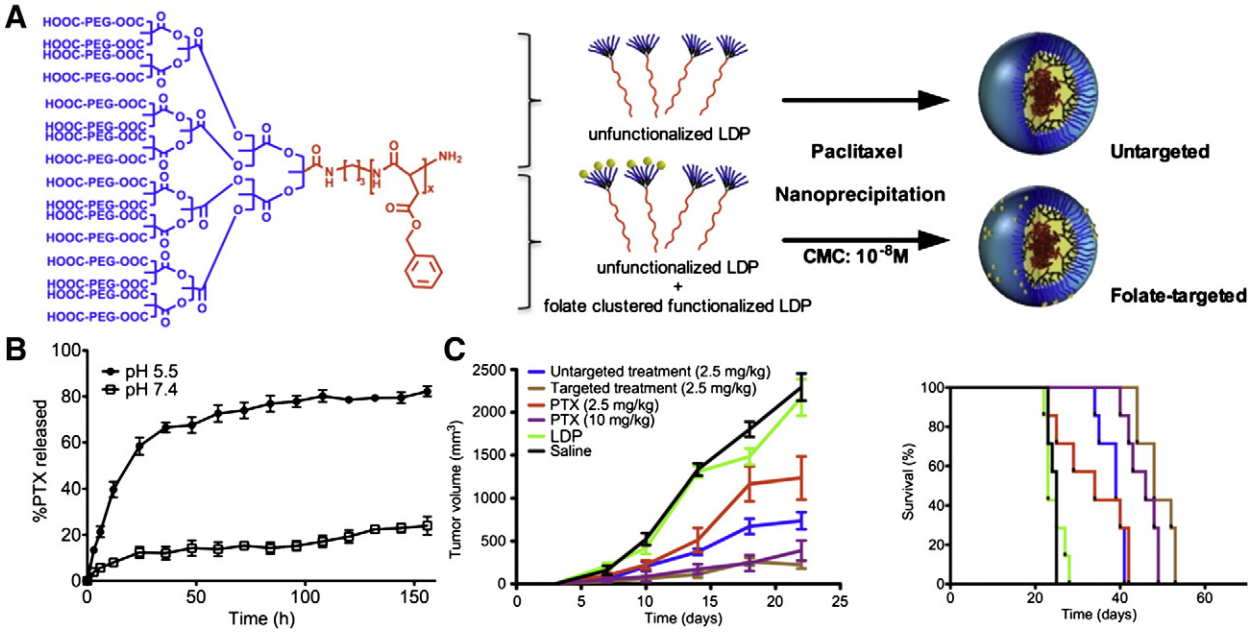

Fig. 4. Folate-functionalized linear dendritic amphiphilic block polymers for targeted delivery of PTX. (A) Chemical structure of the linear dendritic polymers (LDP) made from biocompatible and degradable elements (x = 12–15). Blue, hydrophilic; red, hydrophobic. Schematic showing the preparation of paclitaxel-encapsulated LDP micelles that do not present folate or present folate clusters for enhanced cell targeting; (B) *in vitro* release of PTX at 37 °C in fetal calf serum at pH 7.4 and 5.5 showed that PTX-loaded micelles would be stable in circulation, but the acidic endosomal environment would trigger PTX release; (C) tumor volume and Kaplan–Meier plot of survival times for mice receiving treatments. Antitumor study was tested in nude mice (n = 7) bearing KB xenografts (subcutaneous injection on right flank, day 0). Treatment began on day 4, when tumors were palpable, and groups of mice were given a single-dose intravenous injection (tail vein) on days 4, 8, 12, and 16 of the following: (1) untargeted LDP micelles (100 mg/kg) delivering 2.5 mg/kg PTX; (2) folate-targeted LDP micelles (100 mg/kg) delivering 2.5 mg/kg PTX; (3) 2.5 mg/kg free PTX in 1:1 Cremophor/ethanol (1% vol/vol) as excipient; (4) 10 mg/kg free PTX in 1:1 Cremophor/ethanol (1% vol/vol) as excipient; (5) LDP micelles (100 mg/kg) without PTX; (6) saline. Mice were evaluated over a period of 60 days.
Reprinted with permission from reference [56].

including drug loading capacity, particle size and size distribution, biocompatibility, thermodynamic and kinetic stability. The role of non-covalent interactions is particularly pronounced as a collective driving force for the formation of stable micelles with high drug loading capacity. In this section, the role of hydrogen bonding in the micellar core will be discussed as a means to provide kinetic stability as well as increased drug loading efficiency and capacity (Fig. 1B).

Yang and coworkers demonstrated that the incorporation of hydrogen bonding urea functionalities in the hydrophobic block of amphiphilic diblock copolymers significantly lowered CMC of block copolymers, stabilized the micelles and improved drug loading and facilitated the formation of stable drug-loaded micelles, yet did not induce significant cytotoxicity. The polymers were synthesized through ROP of urea-functionalized cyclic carbonates using mPEG as a macroinitiator. Ureas are known to associate *via* bifurcated hydrogen bonds. Ureas are also known to bind carboxylate derivatives and their isosteres (such as sulfonates, phosphonates, and phosphates), which provides a possible mode of interaction with drug molecules. These findings highlight the importance of the control of non-covalent interactions for supramolecular drug-delivery [17,18]. Since carboxylates and ureas form bifurcated hydrogen bonds, urea- and carboxylic acid-functionalized poly(carbonate) and PEG diblock copolymers (PEG–PUC and PEG–PAC) with narrow molecular weight distributions (polydispersity indices: 1.14–1.20) were synthesized and employed to prepare mixed micelles *via* hydrogen-bonding interactions between urea and acid groups [19] (Fig. 5A). Although PEG–PAC diblock copolymer had high loading capacity for amine-containing DOX (39 wt.%) due to the strong ionic interaction between the acid group in the polymer and the amine group in the drug, the DOX-loaded micelles formed large aggregates having particle size of 595 nm. In addition, they were not stable in the presence of a destabilizing agent (*i.e.* SDS) and most micelles were dissociated in 30 min. In sharp contrast, the mixed micelles formed from PEG–PUC and PEG–PAC were able to load DOX into nano-sized micelles with narrow size distribution at high drug content (166 nm; polydispersity index: 0.18; DOX loading level: 32 wt.%). Importantly, the presence of the urea-functionalized polycarbonate tremendously enhanced the stability of DOX-loaded micelles, and the micelles remained stable even in the presence of SDS and serum proteins over 48 h. The results of *in vitro* release studies showed that DOX release was sustained over 8 h without obvious initial burst release (Fig. 5B) [20]. The DOX-loaded mixed micelles effectively suppressed the proliferation of HepG2 and 4T1 cancer cell lines. The *in vivo* biodistribution studies conducted in a 4T1 mouse breast cancer model with a single i.v. injection of 8 mg/kg DiR-loaded mixed micelles demonstrated that the mixed micelles were preferably transported to the tumor even at 5 days post administration [21]. The concentration of the micelles in the blood after injection was estimated to be about 100 mg/L provided the volume of mouse blood is 1.6 mL, which was much higher than the CMC value of the micelles (16.8 mg/L), suggesting that the micelles would be stable during the blood circulation. This was in good agreement with the findings obtained from the *in vivo* biodistribution study. In the same tumor model, DOX-loaded micelles inhibited tumor growth more effectively than free DOX (percentage of tumor volume: 500% *vs.* 1350% at 26 days post treatment, Fig. 5C) without causing body weight loss (0–15% increase at 26 days post treatment, Fig. 5D) or cardiotoxicity (apoptotic cells in the heart) (Fig. 5E–G) [21].

Another interesting design for enhancing the kinetic stability of micelles is the use of hydrogen bonding that is bolstered by stereo complexation. For example, lactide monomers have two stereoisomers, L- and D-compounds. There are three types of poly(lactide)s: optically active poly(L-(−)-S-lactide) (PLLA) and poly(D-(+)-R-lactide) (PDLA) and racemic poly(D,L-lactide) (PDLLA). It was reported that mixtures of PLLA and PDLA led to the formation of the stereocomplex with a distinctive crystalline structure and morphology [57,58]. Sarasua et al. showed that the stable stereocomplex formation stemmed from H-bonding force from specific CH3···O═C and CαH···O═C interactions

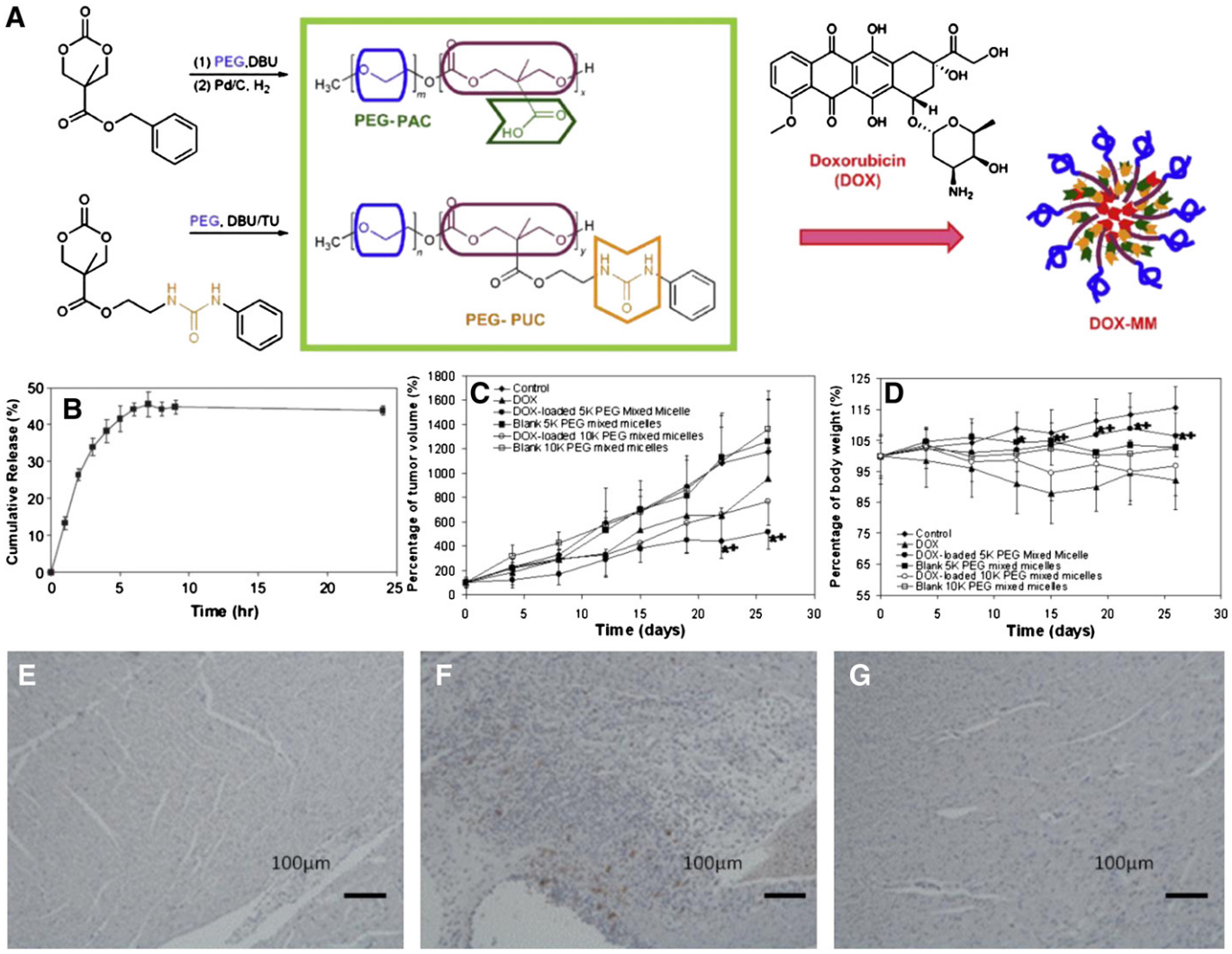

Fig. 5. Polymeric micelles stablized by H-bonds formed between acid and urea groups, which are installed in the hydrophobic blocks poly(carbonate-urea) (PUC) and poly(carbonate-acid) (PAC) of diblock copolymers of PEG-PUC and PEG-PAC, for the delivery of DOX. (A) Synthesis of PEG-PUC and PEG-PAC, and preparetion of DOX-loaded mixed micelles (DOX-MM) formed from PEG-PUC and PEG-PAC. (B) *In vitro* release profiles of DOX-loaded mixed micelles in PBS (pH 7.4) at 37 ° C; (C) tumor volume and (D) body weight changes over 26 days for mice bearing 4T1 tumors administered with PBS (control), free DOX, DOX-loaded 5k PEG and 10k PEG mixed micelles and their respective blank micelles. Percentage of tumor volume or body weight was calculated by dividing the tumor volume or weight at a given time point over the respective values at day 0 and being multiplied by 100%. 5 mg/kg of DOX for free DOX and DOX-loaded mixed micelles and the equivalent weight of blank mixed micelles were given at days 0, 4, 8 and 12. The symbols * and + indicate significant difference in (C) tumor volume or (D) body weight between DOX-loaded 5k PEG mixed micelle-treated and free DOX-treated mice and between DOX-loaded 5k PEG mixed micelle-treated and 10k PEG mixed micelle-treated mice respectively ($p < 0.05$). Histological analysis of hearts at the end of anti-tumor study for TUNEL-positive apoptotic bodies from a representative mouse in each treatment group. Heart sections from a mouse injected with PBS (E); treated with four doses of 5 mg/kg free DOX (F) and four doses of 5 mg/kg DOX-loaded 5 K PEG mixed micelles (G). Many apoptotic bodies are seen in the mouse treated with free DOX, while there are few apoptotic bodies observed in the mouse treated with the DOX-loaded micelles.
Reprinted with permission from reference [20] and [21].

between both PLA stereoisomers from a combined study with FT-IR spectroscopy and molecular modeling [59]. Leroux et al. demonstrated that stereocomplex block copolymer micelles obtained from mixtures of PEG–PLLA and PEG–PDLA exhibited enhanced kinetic stability compared to racemic polymer alone [60]. The kinetic stability of micelles was evaluated by measuring the changes of scattered light intensity of micelles over time in the presense of SDS using dynamic light scattering. The intensity of PEG–PLLA and PEG–PDLA micelles droped dramatically to about 75% after 2 h. In contrast, the intensity of stereocomplex micelles remained more than 80% even after 4 days. The use of stereocomplexation allows a simple and efficient route to yield stabilized mixed micelles capable of tuning their properties and meeting various application requirements such as targeting and stealthness. For example, mixed micelle formation based on a stereoselective association between different stereoisomers of two different block polymers PEG–PDLA and poly(*N*-isopropylacrylamide)–poly(L-lactide) (PNIPAAm–PLLA) in an aqueous environment reduced CMC (5.0 mg/L *vs.* 25.1 mg/L and 7.9 mg/L) and allowed the introduction of both stealthness and a lower critical solution temperature into a single nanostructured micelle [61]. Collaboratively, the groups of O'Reilly and Dove have designed and synthesized block copolymers from poly(ethylene oxide)–poly(benzyl alpha-malate) (both D and L forms), and the mixed micelles from the stereoregular polymers demonstrated significantly lower CMC values relative to the enantiopure forms (5.53 mg/L *vs.* 9.78 mg/L and 12.3 mg/L) [62].

An example highlighting the use of hydrogen-bonding interactions was demonstrated by Tan et al. [63]. Here, three model drugs (*i.e.* ibuprofen, norethisterone and nitrendipine) with different functional groups were dialyzed with dextran-*graft*-poly (*N*-isopropylacrylamide) to form micelles. The polymers were synthesized by grafting PNIPAAm onto dextran using Ce(IV) as a redox initiator [64]. Only ibuprofen was found to produce nanosized drug-loaded micelles, with self-assembly likely driven by the strong hydrogen bonding interactions between the amide groups of hydrophobic PNIPAAm chain and the

carboxylic acid moiety of the drug. In another similar study performed by Zhang et al. [65], seven drugs including ibuprofen (IBU), ketoprofen (KET), naproxen (NAP), indomethacin (IND), dexamethasone (DMS), medroxyprogesterone acetate (MPG) and prednisone acetate (PNS) were loaded into amphiphilic graft copolymer poly (*N*-isopropylacrylamide)/ethyl 4-aminobenzoate-polyphosphazene (PNIPAAm/EAB-PPP) micelles. Drug loadings with IBU, KET, NAP and IND were much higher than the other three drugs (11.2–14.1 wt.% *vs.* 0.3–0.5 wt.%), which was again attributed to favorable hydrogen bonding interactions between the amide groups of PNIPAAm and the carboxylic acid moieties of those drugs. Su et al. designed and prepared the PEG-conjugated multi-arm hyperbranched copolymer, HEHDO-star-mPEG (HEHDO = hyperbranched 5-ethyl-5-hydroxymethyl-1,3-dioxan-2-one), which self-assembled into supramolecular micelles in aqueous solution [66]. HEHDO was synthesized using self-condensing ROP at 120 °C *in vacuo*, and isocyanate-terminated mPEG (mPEG-NCO) was subsequently decorated on the hyperbranched polymer surface to afford the desired material. DOX was loaded into these micelles and stabilized through hydrogen bonding interactions with a high drug loading content (16 wt.%) as well as sustained release *in vitro* (72% released over 48 h).

By introducing some small molecule linkers into the polymeric systems, hydrogen bond formation can be promoted within the micellar cores to enhance stability. Kuang et al. synthesized a nucleobase-grafted amphiphilic copolymer, *i.e.* methoxy poly(ethylene glycol)-*b*-poly(L-lactide-*co*-2-methyl-2(3-(2,3-dihydroxylpropylthio) propyloxycarbonyl)propylene carbonate/1-carboxymethylthymine) (mPEG-*b*-P(LA-*co*-MPT)) for micelle preparation [67]. After adding 9-hexadecyladenine (A-C16), the nucleobase T in the diblock copolymer and A in the A-C16 were found to form strong hydrogen bonding interactions. Not only did the addition of A-C16 significantly decrease the CMC of mPEG-*b*-P(LA-*co*-MPT), but it also enhanced the stability of the micelles in aqueous solution. The *in vitro* drug release profile showed that with the increase of A-C16 content from 0 to 67%, the DOX release rate at pH 7.4 decreased from 18% to 12% over 96 h due to the strong hydrogen bonding interaction. At the same time, DOX release became much faster at pH 5.0 due to the increased solubility of DOX at pH 5.0 and also the fact that protonation diminishes the hydrogen bonding effect. Another approach was based on using phenol–pyridine hydrogen bonding interactions to prepare core–shell micelles [68]. In one example, two diblock copolymers poly(styrene-*b*-4-vinylphenol) and poly(styrene-*b*-4-vinylpyridine) were synthesized and mixed with the small-molecule hydrogen-bonding crosslinkers bis-pyridylethane and bisphenol A, respectively. The diblock copolymers formed hydrogen bonds with the crosslinkers *via* phenol–pyridine interactions, resulting in micelles that were more stable than the ones without crosslinking agents.

The hydrogen bonding interactions that help stabilize micelles can be affected by both the polymer compositions and drug structures. In the urea-functionalized copolymer system, increasing the proportion of urea groups from 0 to 40 mol% in the hydrophobic block was observed to reduce CMC from 11.2 to 2.8 mg/L while decreasing the micelle size from 360 to 111 nm and increasing drug loading level of DOX-loaded micelles from 6.9 to 10.3 wt.%. This is due to the larger proportion of urea groups resulting in a greater degree of hydrogen bond interactions within the micellar core, with one another and also with the loaded DOX [18]. In another study, six drugs, namely IBU, KET, IND, DMS, MPG and PNS, were loaded into amphiphilic graft copolymer poly(*N*-isopropylacrylamide)/ethyl tryptophan-polyphosphazene (PNIPAAm/EtTrp-PPP) micelles. The drug loadings associated with IBU, KET and IND were found to be much higher than the other three drugs (0.9–13 wt.% *vs.* 0.2–1.3 wt.%) [69]. One of the main reasons is because of the strong hydrogen bonding interactions between the amide groups in PNIPAAm chains and the carboxylic acid groups in IBU, KET and IND. The results also showed that a higher proportion of PNIPAAm chains led to a higher drug loading of IND.

4. Ionic interaction

Ionic interactions are long-range interactions that involve the electrostatic attraction between oppositely-charged ions, *i.e.* cations (positive) and anions (negative). Ionic interactions have been widely employed as a tool to form micelles for drug delivery. Compared with micelles that are self-assembled through hydrophobic and hydrogen bonded interactions, micelles formed by ionic interactions have the added advantage of encapsulating ionic compounds such as small molecule drugs, therapeutic proteins, peptides and nucleic acids. Within the micelles, ionic interactions can occur between two oppositely-charged polymer chains or between oppositely-charged functional groups on the polymers and on the loaded compound (Fig. 1C). Some commonly reported systems include polyionic complex (PIC) micelles self-assembled from block copolymers comprised of a polyionic segment and a hydrophilic segment. These are assembled primarily through ionic interactions, and are able to encapsulate drugs within the core for subsequent delivery. In our previous review article, we provided an account of PIC mixed micelles composed of two oppositely-charged copolymers [6]. Here, we will only focus on examples of micelles formed from a single copolymer. The ionic interactions between the copolymer and the loaded compounds, and the various factors that influence these interactions, will be discussed.

Ionic interactions have been widely used as a tool for preparing stable PIC micelles for encapsulating charged small molecule drugs. For instance, all-*trans* retinoic acid (ATRA) in its deprotonated form is an anionic drug that can be loaded into the cationic copolymer poly(ethylene glycol)-*graft*-chitosan to form PIC micelles that are stabilized by ionic interactions [6,70]. The drug loading efficiency was higher than 80% and the drug-loaded micelles showed more sustained drug release *in vitro* than the free drug. Yang et al. prepared PIC micelles based on methoxy poly(ethylene glycol)-*graft*-chitosan (mPEG-*g*-Chitosan) and lactose-conjugated PEG-*graft*-chitosan (Lac-PEG-*g*-Chitosan) for the delivery of the anionic drug diammonium glycyrrhizinate (DG) [71]. The drug loading efficiency of DG-loaded regular PIC micelles and lactose-modified PIC micelles were 97.4% and 96.7%, respectively. The two micelles were stable in acetate buffer (pH 3.5, 1%) for 3 months without aggregation. Just as anionic drugs can be loaded into polycations, cationic drugs can also be loaded into anionic copolymers to form PIC micelles. For example, a cationic drug imipramine hydrochloride was loaded into a four-arm poly(ethylene oxide)-*b*-poly(methacrylic acid) block copolymer through the ionic interaction between the negatively-charged carboxylate groups on the polymer chains and the positively-charged imipramine hydrochloride [72]. Eckman et al. designed and synthesized the block copolymer PEG–poly(aspartate) (PEG–p(Asp)) to form PIC micelles with the cationic drug DOX hydrochloride [73]. Carboxyl groups of p(Asp) were present as benzyl ester (PEG–p(Asp/Bz)), sodium salt (PEG–p(Asp/Na)) or free acid (PEG–p(Asp/H)). The drug loading of PEG–p(Asp/Na) and PEG–p(Asp/H) micelles were much higher than that of PEG–p(Asp/Bz) micelles (56.8 wt.% and 40.6 wt.% *vs.* 1.1 wt.%) due to the strong ionic interactions within the PEG–p(Asp/Na) and PEG–p(Asp/H) micelles. Among the three micelles, PEG–p(Asp/Na) micelles were the most stable *in vitro*, facilitating the gradual release of drug over a prolonged period for the effective suppression of cancer cell growth (68.2% released over 48 h). In these drug molecules, there are hydrophobic components, which may also play a role in drug loading within a micelle.

Proteins and peptides contain many charged moieties and may show an overall negative or positive charge in different pH depending on their characteristic pI (isoelectric point) values. Their charged nature makes them suitable for encapsulation into micelles *via* ionic interactions. For example, we reported on the loading of the anticancer protein lectin A-chain into cationic micelles based on biodegradable and amphiphilic copolymers [74]. The lectin A-chain loaded cationic micelles showed much smaller particle sizes and stronger positive charges than the commercial product BioPorter and lectin A-chain complexes (150 nm *vs.*

455 nm; +30 mV *vs.* +20 mV). Consequently, the cationic micelles exhibited much higher delivery efficiency than BioPorter in various cancer cell lines. In MDA-MB-231 human breast cancer, HeLa human cervical cancer, HepG2 human liver carcinoma and 4T1 mouse breast cancer cell lines, IC50 of lectin A-chain/cationic micelle complexes was 0.2, 0.5, 10 and 50 mg/L, respectively, while that of BioPorter and lectin A-chain complexes was higher than 100 mg/L in all cell lines tested. Besides cationic micelles, PIC micelles self-assembled through the ionic interaction between proteins/peptides with the charged segment of PEG-based copolymers, can also be formed to improve drug loading capacity and kinetic stability. Kataoka and coworkers reported core–shell-type PIC micelles formed by lysozyme and PEG–P(Asp) [75]. These micelles had an extremely narrow size distribution with an average diameter of 47 nm. Wang et al. prepared PIC micelles based on methoxy poly(ethylene glycol)-*grafted*-chitosan (mPEG-*g*-chitosan) and a targeting peptide Arg-Gly-Asp (RGD)-conjugated poly(ethylene glycol)-*graft*-chitosan (RGD-PEG-*g*-chitosan) for targeted delivery of recombinant hirudin variant-2 (rHV2) towards platelets [76]. Both of these PIC micelles showed high drug encapsulation efficiencies (76.90 ± 0.84% and 81.08 ± 0.85%) and enhanced stability compared with rHV2 solution *in vivo* (mean retention time: 207.4 ± 19.2 min and 198.1 ± 5.2 min *vs.* 160.7 ± 7.7 min) [76]. Kataoka et al. reported a novel protein delivery system for intracellular delivery based on charge-conversional PIC micelles. A copolymer PEG–poly(*N*-(*N*′-(2-aminoethyl)-2-aminoethyl)aspartamide) (PEG–pAsp(EDA)) bearing primary amines on the side chains was modified with citraconic anhydride (Cit) to afford the negatively-charged copolymer PEG–pAsp(EDA-Cit) with pendant carboxylate groups. This polymer formed PIC micelles when combined with lysozyme, a positively-charged protein, at neutral pH. Upon endocytosis and subsequent uptake into the endosome, the acid-promoted degradation of the citraconic anhydride resulted in a change in overall charge of the copolymer from negative to positive, which consequently led to the dissociation of the PIC micelles [77]. In a follow-up study, a positively-charged protein immunoglobulin G (IgG) was modified with citraconic acid amide (Cit) or *cis*-aconitic acid amide (Aco) to convert the surface charges of IgG from positive to negative (Fig. 6A). IgG modified with succinic anhydride (Suc) was synthesized as negative control, which does not degrade under acidic pH conditions. The modified protein, now negatively-charged, formed PIC micelles with the positively-charged copolymer

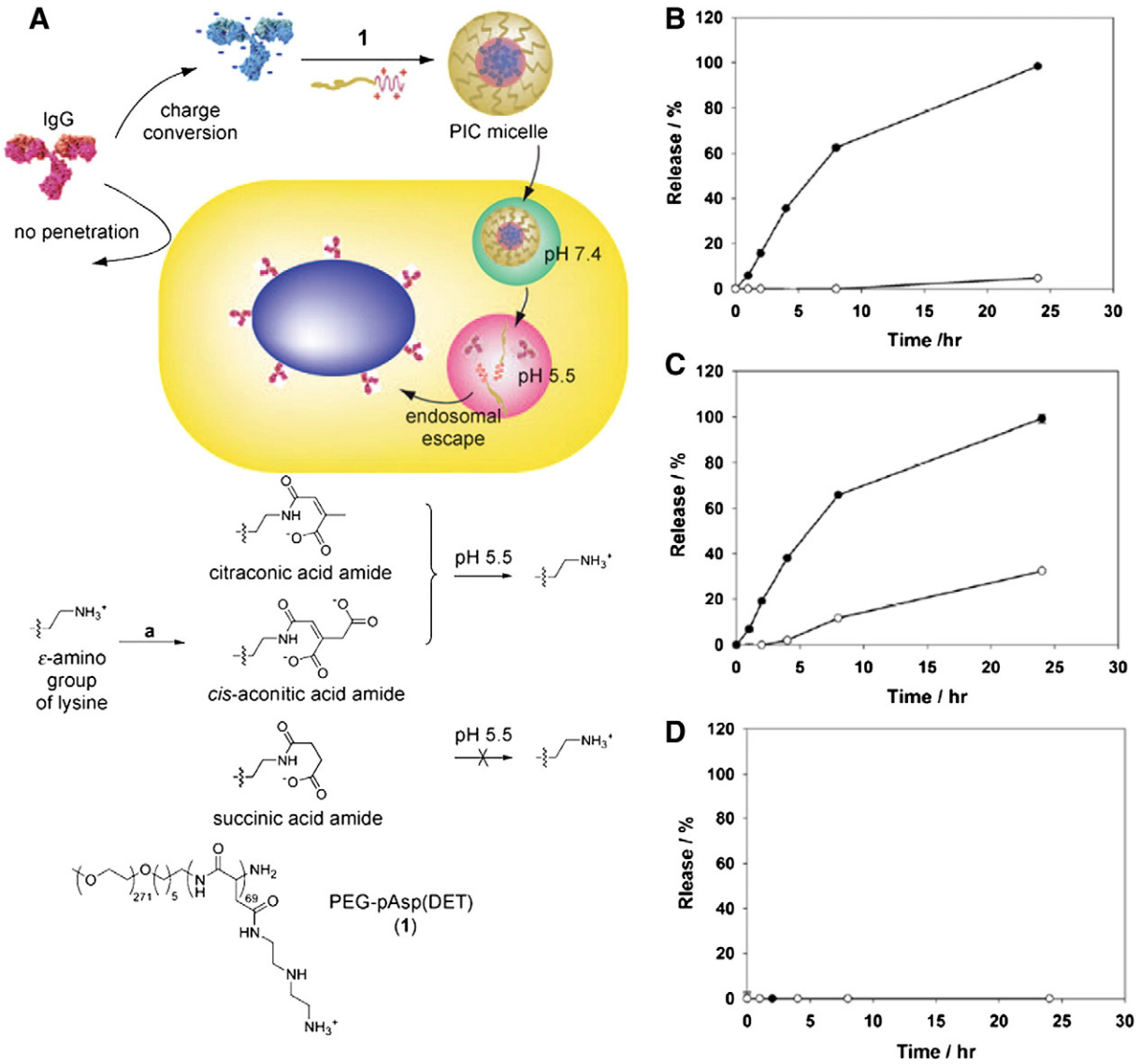

Fig. 6. Preparation of the charge-conversional PIC micelles between IgG derivatives and PEG–pAsp(DET) (A) and *in vitro* release profiles of the Alexa Fluor 488-labeled (Fab′)2 fragment derivatives from the PIC micelles containing (B) (Fab′)2-Cit, (C) (Fab′)2-Aco, and (D) (Fab′)2-Suc at 37 °C, pH 5.5 (●) and pH 7.4 (○).
Reprinted with permission from reference [78].

PEG–pAsp(DET). Similarly, under low pH conditions within the endosome, the degradation of citraconic acid amide or *cis*-aconitic acid amide resulted in the surface charge of the protein changing from negative to positive, leading to the release of IgG (Fig. 6B and C). The PIC micelles containing succinic anhydride modified IgG did not release IgG at either pH 7.4 or pH 5.5 (Fig. 6D) [78].

By virtue of the overall negative charge on nucleic acids, ionic interactions represent the key driving force in facilitating their incorporation into micelles with improved stability *in vitro* and *in vivo* [79]. Yang and coworkers had previously designed and synthesized a cationic amphiphilic copolymer P(MDS-*co*-CES) containing tertiary amine groups for intracellular gene delivery *via* the "proton-sponge" effect [46]. The copolymer self-assembled into cationic micelles with a CMC value of 10 mg/L. These micelles effectively delivered DNA and siRNA into various human cancer cell lines, and showed lower toxicity and higher transfection efficiency than polyethylenimine (PEI, 25 kDa) [80,81]. In addition, they also reported cationic micelles self-assembled from amphiphilic oligopeptides and used them for gene delivery [82,83]. The use of these micelles resulted in higher gene expression efficiency compared to peptide carriers that did not form micelles. Typically, the PIC micelles were self-assembled from copolymers with a polyionic segment and PEG as the hydrophilic segment. For example, Yang and coworkers synthesized a copolymer folate–poly(ethylene glycol)-*graft*-chitosan (FA–PEG–Chi) for targeted plasmid DNA delivery to tumor cells [84]. The copolymer and DNA formed PIC micelles composed of a PEG shell and a core of chitosan and DNA held together by ionic interactions. Kataoka et al. reported a copolymer poly(ethylene glycol)-*b*-poly(L-lysine) comprising lysine amines modified with 2-iminothiolane (2IT) at 95% and cyclo-RGD (cRGD) for the targeted delivery of siRNA to solid tumors [85]. The use of 2IT allowed for disulfide cross-linking in the core, and increased the hydrophobicity of polymer, leading to enhanced micelle stability. This system produced PIC micelles that afforded increased gene silencing ability, improved cellular uptake, broader subcellular distribution *in vitro*, and also improved accumulation in both the tumor mass and tumor-associated blood vessels when injected intravenously into HeLa tumor-bearing mice compared with naked siRNA and micelles without 2IT or RGD modification. More recently, Qian et al. prepared a targeting peptide TGNYKALHPHNG (TGN)-modified PEGylated poly(2-(dimethylamino) ethyl methacrylate) (TGN-PEG-PDMAEMA) copolymer for DNA delivery [86]. The TGN-modified PIC micelles showed good DNA condensation capacity, low toxicity, and increased cellular uptake compared with the unmodified polyplexes (PEG-PDMAEMA/DNA polyplexes).

The ionic interactions within the micelles can be modulated by pH. As pH increases, the positively-charged copolymer or cationic drug cargo will become less ionized and eventually neutralized. Conversely, negatively-charged copolymers or drug molecules will become less ionized as pH decreases. In the DOX-loaded PEG–p(Asp) block copolymer PIC micelles, the release kinetics of DOX from PEG–p(Asp/Bz) micelles were unaffected by pH (*i.e.* identical at pH 7.4 and 5.0) as the PEG–p(Asp/Bz) micelles are assembled and stabilized through hydrophobic rather than ionic interactions [73]. In contrast, for micelles such as (PEG–p(Asp/Na)) and (PEG–p(Asp/H)) that are assembled by ionic interactions, the release of DOX was much faster at pH 5.0 than at 7.4 (90% *vs.* 70–75% released over 48 h), whereby the lower pH neutralizes the negative charges of the copolymer, weakens the ionic interactions, and consequently destabilizes the micelles. Lastly, for the charge-conversional PIC systems mentioned above, micelle stability is maintained at neutral pH, whereas decreasing the pH to 5.5 led to the conversion of charges and subsequent weakening of the ionic interactions [77].

Another factor that is also known to influence the ionic interactions within micelles is the length of charged segment of the copolymer. For instance, complexes between DNA and a block copolymer comprised of poly-L-lysine and PEG (PLL–PEG) blocks were prepared and tested in *in vivo* turnover studies [87]. The PIC micelles with a longer PLL chain length (48-mer) showed greater stability in the blood than those with a shorter PLL chain length (19-mer) due to the larger degree of ionic interactions. Loh et al. prepared a series of amphiphilic copolymers with poly(dimethylaminoethyl methacrylate) (PDMAEMA) and poly(propylene glycol methacrylate) (PPGMA). The hydrophobic PPGMA interior allowed for a cell-sensitizing drug to be incorporated, while the cationic and hydrophilic PDMAEMA corona was able to complex with DNA to form a nano-sized polyplex [88]. The agarose gel electrophoresis test showed that the higher PDMAEMA content in the polymer increased the ability of the cationic polymer to form polyplexes with DNA (polymer with 84.0% PDMAEMA condensed DNA at N/P ratio of 2, while polymer with 23.4% PDMAEMA condensed DNA at N/P ratio of 7), likely due to stronger ionic interactions.

Finally, it should also be noted that the strength of ionic interactions within PIC micelles can also be affected by the presence of other ions in solution. As ion concentration increases, the charged copolymer or loaded cargo will interact electrostatically with the ions in solution, leading to the weakening or breakup of ionic interactions within PIC micelles. In a study by Kataoka and co-workers, isothermal titration calorimetry (ITC) was used to explore the complexation process between DNA molecules and PEG–PLL [89], whereupon it was found that the binding between DNA and the copolymer was stabilized as the salt concentration decreased.

5. Chemical cross-linking

Conventional micelles exist in solution only at concentrations higher than their CMC, below which they become thermodynamically unstable and spontaneously disintegrate, leading to premature drug release. This represents a major obstacle for intravenous delivery applications, as the micelle experiences infinite dilution as soon as it is injected into the bloodstream. To overcome this limitation, cross-linking strategies have been explored to enhance micelle stability. Various strategies have been developed for the preparation of cross-linked micelles and a range of stimuli-sensitive linkages have been introduced into these systems to achieve on-demand drug release to targeted sites [90,91]. Based on the location of the cross-linking, cross-linked micelles can be categorized into core cross-linked, shell cross-linked and intermediate layer cross-linked. Fig. 1D illustrates formation of a core cross-linked micelle. In this section, we will discuss the chemical cross-linking interactions within these three types of micelles.

5.1. Core cross-linking

Cross-linking of the micelle core has been proven to enhance micelle stability and prevent destabilization upon dilution. For example, Shuai et al. reported core cross-linked (CCL) polymeric micelles for PTX delivery [92]. The multistep polymer syntheses involved ROP of ε-caprolactone (CL) initiated by mPEG in the presence of tin(II) octanoate followed by end-capping with a reactive maleic moiety; subsequent esterification of the acid-terminated intermediate with mPEG–PCL gave the desired triblock copolymer, mPEG–PCL–mPEG, in greater than 95% yield. The micelles were then cross-linked by radical polymerization *via* the double bond of the maleic group. Compared with non-cross-linked micelles, the CCL micelles exhibited a significantly enhanced thermodynamic stability and PTX-loading efficacy. Several elegant studies have reported on the use of pH-sensitive core cross-linked micelles for loading and release of various hydrophobic drugs [93–96]. In one example, Zhong et al. developed core cross-linked, pH-sensitive micelles based on poly(ethylene glycol)-*block*-poly(mono-2,4,6-trimethoxybenzylidene-pentaerythritolcarbonate-*co*-acryloyl carbonate) (PEG-*b*-P(TMBPEC-*co*-AC)) diblock copolymers [95] (Fig. 7A). After photo cross-linking, the core cross-linked micelles displayed high stability at pH 7.4 (*e.g.*, pH of the extracellular environment), while the micelles underwent rapid degradation *via* hydrolysis at acidic pH of 4.0 and 5.0 (mimicking the endo/lysosomal compartments).

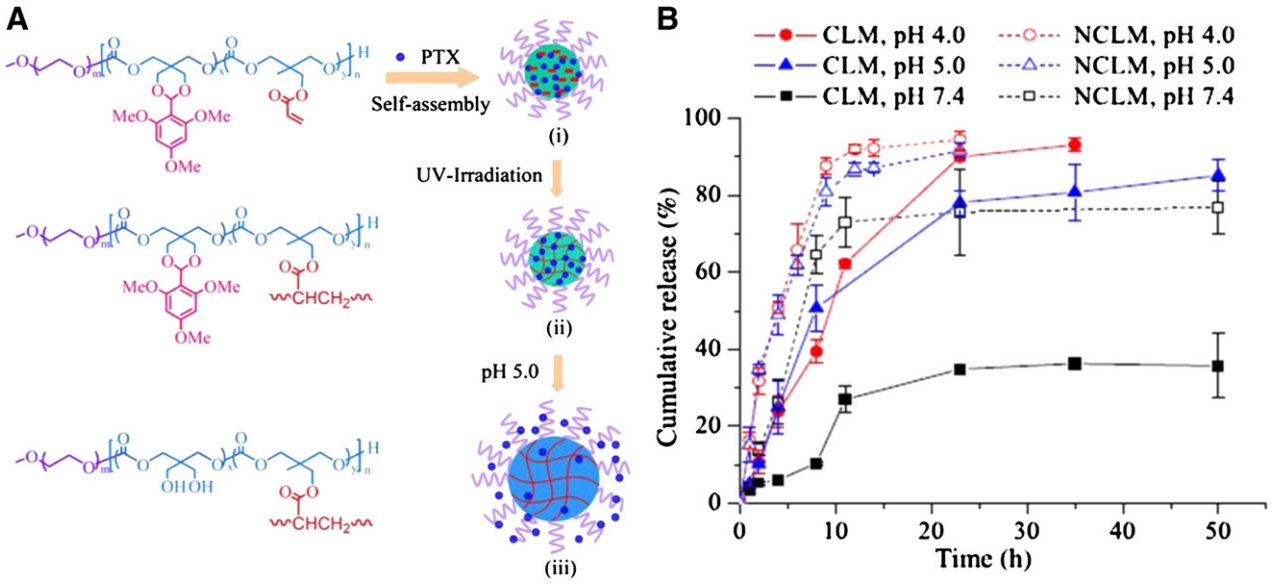

Fig. 7. Illustration of photo-cross-linkable pH-sensitive degradable micelles based on PEG-*b*-P(TMBPEC-*co*-AC) block copolymer (A) and pH-dependent drug release from PTX-loaded cross-linked micelles at 37 °C (B). The release profiles of PTX from non-cross-linked micelles were used as control. PTX-loaded cross-linked pH-sensitive degradable micelles exhibited superior extracellular stability while "actively" releasing PTX under the acidic condition mimicking that of the endo/lysosomal compartments.
Reprinted with permission from reference [95].

Release of PTX from the aforementioned micelles *in vitro* was greatly inhibited at pH 7.4 (33% *vs.* 75% over 23 h) due to the cross-linked nature of the micellar core (Fig. 7B). Notably, however, rapid drug release was observed under acidic conditions, in which 90.0% and 78.1% of the loaded PTX was released in 23 h at pH 4.0 and 5.0, respectively (Fig. 7B). In another study, Lin et al. reported the use of amphiphilic poly(aspartamide) (P(Asp)) copolymers for the synthesis of core cross-linked micelles. These P(Asp) polymers were fabricated by grafting NH_2-terminated mPEG(5k), 1-(3-aminopropyl)imidazole, and cinnamate onto the polysuccinimide backbone by sequential substitution. Cross-linking was subsequently accomplished by photopolymerization of the cinnamate groups. After cross-linking, the micelles exhibited higher stability over a wider pH range and displayed a characteristic pH-dependent swelling and shrinking behavior in contrast to the micelle–unimer transition behavior exhibited by the non-cross-linked micelles. PTX was effectively loaded into the micelles with 15 wt.% loading level, which in the case of the cross-linked system exhibited a prolonged release of the drug at both high and low pH in comparison to the burst release exhibited by the non-cross-linked micelles (20% *vs.* 50% at pH 7.4 over 48 h and 52% *vs.* 88% at pH 5.0 over 8 h) [96].

The drastic concentration gradient of glutathione in the intra- *vs.* extra-cellular environment has also been widely exploited for the design of redox-responsive micelles. A popular approach for fabricating such redox-responsive micelles involves the use of disulfide linkages as micellar core (or shell) cross-linking agents, which can subsequently be cleaved in a reductive environment such as the interior of a cell, thereby releasing the payload [97–100]. In one example, Zhong et al. reported reversible redox-responsive core cross-linked micelles based on poly(ethylene glycol)-*b*-poly(*N*-2-hydroxypropyl methacrylamide)-lipoic acid conjugates and investigated their use for triggered DOX release [98]. The *in vitro* release results showed that only about 23.0% of DOX was released in 12 h from cross-linked micelles at 37 °C, whereas about 87.0% of DOX was released in the presence of 10 mM dithiothreitol (DTT) under otherwise similar conditions. Li et al. reported PTX-loaded reversible disulfide core cross-linked micelles (PTX-DCMs) formed from the self-assembly of thiolated telodendrimers and cross-linked by the oxidization of thiol groups to disulfide bonds [99] (Fig. 8A). Cross-linking of the micelles within the core was hypothesized to decrease their CMC values and markedly enhance their stability post administration and in non-reductive physiological environments. The PTX release from the disulfide cross-linked micelles was significantly slower than that from PTX-loaded non-cross-linked micelles (PTX-NCMs) (10% *vs.* 18% over 5 h), but was enhanced by adding the reducing agent glutathione (GSH) or *N*-acetylcysteine (NAC) (Fig. 8B–E). The blood circulation times of vehicle and payload were investigated by conjugating BODIPY with the polymer and loading DiD within the micelles, respectively. The results showed that the BODIPY signal of NCMs was rapidly eliminated and fell to the background level within 8 h post-injection, while that of DCMs was sustained up to 24 h. Similarly, DiD signal of NCMs decreased faster in spite of the initial increase while that of the DCMs remained up to 30 h. The toxicity of blank micelles was tested in nude mice. At a single dose of 400 mg/kg, all the mice treated with NCMs died within 2 h. On the contrary, none of the mice died in the DCM-treated group at the same dose. The *in vivo* anti-tumor activity was further evaluated in the subcutaneous human ovarian SKOV-3 tumor bearing mice. At a dose of 10 mg/kg, PTX-DCMs showed superior tumor growth inhibition and longer survival time than PTX-NCMs (median survival time: 28.5 days *vs.* 32.5 days). The anti-tumor activity of PTX-DCMs was further improved when combined with NAC. In a follow up study, these disulfide core cross-linked micelles were also used to encapsulate another anticancer drug, vincristine [100]. The drug-loaded cross-linked micelles exhibited superior antitumor activity, upon addition of the reducing agent NAC, than the free drug in lymphoma xenografted nude mice ($p < 0.05$). In a separate report, Yan et al. synthesized a block polymer mPEG-*b*-P(LA-*co*-MTC_{SH}) through ROP of a cyclic carbonate monomer, 2-(2,4-dinitrophenylthio) ethyl-5-methyl-2-oxo-1,3-dioxane-5-carboxylate (MTC_{SH}) and L-lactide (LA) using mPEG as a macroinitiator and deprotection for the preparation of disulfide cross-linked micelles [101]. DOX was loaded as a model drug. The CCL micelles exhibited enhanced stability against the disruptive conditions where the micelle solution was diluted with an organic solvent (dimethylformamide) compared with the non-cross-linked micelles. Apart from the examples mentioned above, micelles with hydrophilic shells and cross-linked polyionic cores have also been reported [102–104]. For instance, Kim et al. developed polymeric micelles synthesized from block ionomer complexes (BIC) of poly(ethylene oxide)-*b*-poly(methacrylic acid) (PEO-*b*-PMA), which was core cross-linked using a biodegradable disulfide, cystamine, and loaded with 50

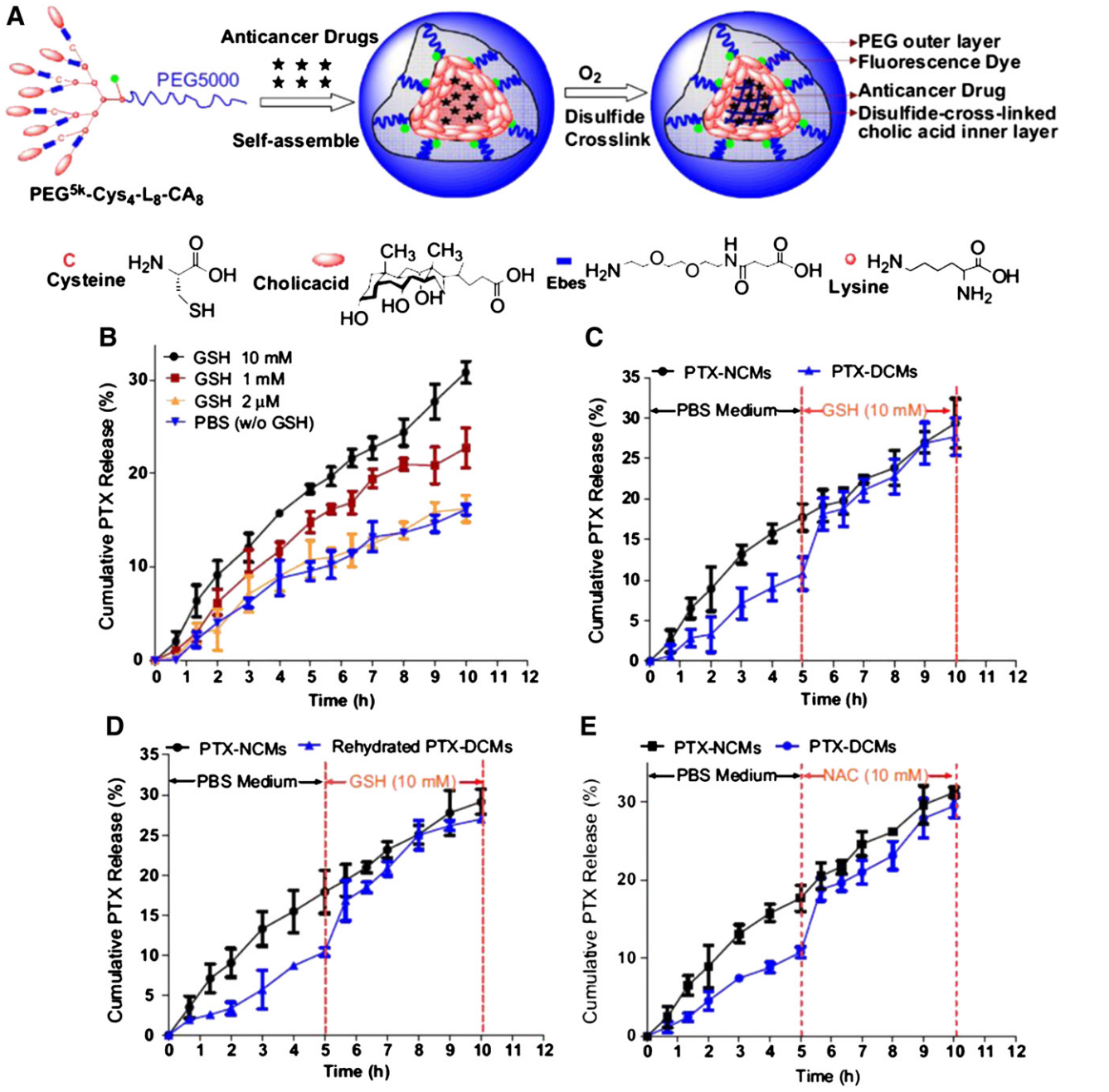

Fig. 8. Schematic representation of the PTX-loaded disulfide cross-linked micelles formed by oxidization of thiolated telodendrimer PEG5k-Cys4-L8-CA8 after self-assembly (A) and PTX release profiles of PTX-DCMs at different GSH concentrations (B); GSH-responsive PTX release profiles of freshly prepared PTX-DCMs (C) and re-hydrated lyophilized PTX-DCMs (D) by adding GSH (10 mM) at a specific release time (5 h) comparing with PTX-NCMs; NAC-responsive PTX release profiles of PTX-DCMs (E) by adding NAC (10 mM) at a specific release time (5 h). Values reported are the mean values ± SD for triplicate samples.
Reprinted with permission from reference [99].

wt.% of DOX. The micelles were highly stable after cross-linking and showed reduction-sensitive drug release profiles [105].

While the major focus of CCL micelles is their use as small molecule drug carriers, a few studies have also reported on the use of such micelles for loading of nucleic acids to enhance their stability and transfection efficiency. For example, Kataoka's group synthesized thiolated c(RGDfK)-poly(ethylene glycol)-*b*-poly(lysine) block polymers for the preparation of disulfide cross-linked polyplex micelles through ion complexation with pDNA [106]. RGD was conjugated onto the micelle surface for active targeting. The resulting polyplex micelles achieved 20-fold higher transfection efficiency in HeLa cells compared with the micelles without RGD and cross-linking. A similar work was reported by the same group in 2009 [107]; in this study, a core–shell-type PIC micelle with a disulfide cross-linked core was prepared through the assembly of 2IT-modified poly(ethylene glycol)-*block*-poly(L-lysine) and siRNA. These CCL micelles maintained micellar structure even at a NaCl concentration as high as 600 mM. In contrast, the non-cross-linked micelles were not stable under the same conditions. The *in vitro* transfection results showed that the CCL micelles achieved 100-fold higher transfection efficacy compared with non-cross-linked PICs, due to their enhanced stability.

5.2. Shell cross-linking

Shell cross-linked (SCL) micelles consist of amphiphilic copolymers that self-assemble to form a hydrophobic core and hydrophilic shell, the latter of which is chemically cross-linked, usually in a step following

micelle formation. The crosslinking of the micellar shell can enhance the stability of micelles under environmental variations, such as ionic strength, solvent system, and pH, and can also affect the drug loading capacity and drug release profile. Kim et al. reported preparation of SCL micelles as carriers for albendazole by using poly(ethylene glycol methyl ether methacrylate-*co*-methacrylic acid)-*block*-poly(methyl methacrylate) block copolymer [108]. Shell cross-linking using 1,8-diaminooctane significantly increased the micelle stability in cell culture media and had a major effect on the rate of drug release, dramatically reducing the amount of drug released from 50% (non-cross-linked) to around 20% (cross-linked) over a 30 h incubation period. Wei et al. described the preparation of SCL micelles self-assembled from a poly(*N*-isopropylacrylamide-*co*-3-(trimethoxysilyl) propylmethacrylate)-*b*-poly(methyl methacrylate) copolymer [109]. Compared with non-cross-linked micelles, the SCL micelles exhibited much higher prednisone acetate entrapment efficiency (EE) as well as lower release rate *in vitro* (EE: 32.0% *vs.* 11.5%; time to reach 80% release: 213 h *vs.* 10 h) compared with the corresponding non-cross-linked micelles. Wooley et al. have devoted significant efforts in the pursuit of shell/core cross-linking nanoparticles for biomedical applications [91,110–112]. In a recent well-designed example, they synthesized a series of polyphosphoester (PPE)-based cross-linked micelles with anionic, cationic and zwitterionic surfaces in order to systematically probe their corresponding stability and toxicity [113]. In general, they found that cationic nanoparticles were less stable compared to their zwitterionic counterparts, which were in turn less stable than the anionic micelles. These novel cross-linked micelles exhibited lower cytoxicity against RAW 264.7 mouse macrophages relative to commercially-available materials such as Lipofectamine, PEI and Cremophor, and their degradation products were not detrimental to the cells tested.

5.3. Intermediate layer cross-linking

While core and shell cross-linking have been shown to impart improved stability upon micelles when compared against their non-cross-linked counterparts, several disadvantages exist for these systems. For instance, synthesis of shell cross-linked micelles often results in unintended intermicellar cross-linking, an unwanted side-reaction that requires tedious optimization in order to avoid [90]. Furthermore, shell cross-linking also reduces the fluidity and hydrophilicity of the micelle. Core cross-linking, on the other hand, can limit the mobility of the micellar core as well as its drug loading capacity. Intermediate layer cross-linked micelles have been hypothesized to alleviate these issues [114]. Recently, Desale et al. synthesized triblock copolymers containing blocks of poly(ethylene glycol), polyglutamic acid and polyphenylalanine (PEG–PGlu–PPhe) to form intermediate layer cross-linked micelles comprised of a PPhe hydrophobic core, a cross-linked ionic PGlu intermediate shell layer, and a PEG corona [115]. These micelles incorporated a combination of two drugs, cisplatin and PTX (cisplatin loading level: 15 wt.%; PTX loading level: 9 wt.%), and the resultant dual drug-loaded micelles demonstrated synergistic cytotoxicity against human ovarian A2780 cancer cells, exemplified by enhanced antitumor activity compared with cisplatin-loaded micelles, PTX-loaded micelles or free cisplatin in an ovarian A2780 cancer xenograft mouse model (survival time: 45 days *vs.* 22 days, 16 days and 20 days respectively). Although presently reports on successful drug loading using such intermediate layer cross-linking methods are still limited, this area would be fascinating to explore in the future to unleash the full advantages of cross-linked micelle systems for drug delivery.

6. Polymer–drug conjugation

Micelles assembled by polymer–drug conjugates have been extensively explored as emerging drug delivery systems over the past decades [2,116]. Their micellar self-assembly in aqueous solution helps improve solubility, increase payload and enhance stability of the therapeutics (Fig. 1E). To avoid premature cargo release in the blood stream and achieve rapid drug release in target sites, the drugs are linked to the polymeric backbone through bonds that are responsive to environmental or physiological stimuli, such as the lower pH in tumor tissue, reducing environment in cells, or temperature change, to achieve a modulated drug release. Furthermore, various targeting ligands can be decorated on the micellar surfaces to attain active targeting [2]. The primary aim of this section is to provide an overview of polymer–drug conjugate systems that are of clinical relevance [1,117]; we shall focus on three major polymeric platforms including PEG, poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) and poly(glutamic acid) (PGA), as well as a few recent examples on polymer–drug conjugate containing biodegradable polymeric backbone. We will also briefly describe and discuss the various chemical conjugation methodologies and their subsequent dissociation for effective release of the active therapeutics.

PEG is a linear non-biodegradable polyether that is industrially manufactured from ethylene oxide in the presence of an acidic or basic catalyst. It is a FDA-approved, hydrophilic polymer that is biocompatible and non-toxic to the human body. Several anticancer PEG–drug conjugates are currently in clinical trials [117,118], including PEG-SN38 (SN38 = 7-ethyl-10-hydroxy-camptothecin; EZN-2208) [119,120], PEG-CPT-11 (CPT-11 = irinotecan (semisynthetic analog of camptothecin); NKTR-102) [118] and PEG-DTX (DTX = docetaxel; NKTR-105) [118]. Many such conjugates utilize linker chemistry for assembling the drug and PEG onto a common platform. Typically, the hydroxy-terminus of the PEG is modified into a carboxylic acid moiety using a small molecular linker, thus allowing ester-forming reaction to occur between the alkoxy-group of the respective drug [118,121]. The construction of NKTR-102, NKTR-105 and EZN-2208 prodrug systems were all derived using a 4-arm PEG platform, which were reported by Nektar Therapeutics and Enzon Inc., separately. Ester-linkages are susceptible to cleavage by esterases and changes in pH, triggering C–O bond hydrolysis that results in drug release from the polymeric backbone although the linkages may take a long time to degrade. Notably, PEG–CPT (CPT = camptothecin; PROTHECAN) and PEG–PTX were recently suspended from phase II (gastro-esophageal adenocarcinoma) and phase I (solid tumor) clinical trials, respectively [118]. An in-depth review discussing the clinical development of PEG–drug conjugates has been reported [118].

PHPMA is another hydrophilic polymer that can be used in lieu of PEG. Importantly, it is non-immunogenic and non-toxic although it is also considered non-biodegradable. PHPMA–DOX was the first polymer–drug conjugate system to enter clinical trial in 1994 [122], and there has been significant progress made towards clinical applications since then. Besides CPT and PTX [123], PHPMA has also been employed in the chemical conjugation to DOX (PHPMA-DOX (PK1/FCE-28068) [122,124,125], PHPMA-DOX-Gal (Gal = galactose; PK2/FCE-28069) [124,126]) and platinum-based drugs (PHPMA-DACH-platinate (DACH = diaminocyclohexane; AP5346/ProLindac™) [127–129] as well as PHPMA-malonato-platinate (AP5280) [130,131]). Both DOX conjugates were synthesized *via* amide-bond formation using oligopeptidic side chain linkers; these labile linkers were designed to be the main site of cleavage by cysteine proteases, required for the consequential drug release. On the other hand, the platinate conjugates were constructed using platinum coordination to PHPMA's oligopeptidic side-chains containing malonato-amido ligands in both AP5346 and AP5280. AP5346 and AP5280 are close macromolecular-mimetics of oxaliplatin and carboplatin, containing one diaminocyclohexane ligand and two ammonia (NH_3) ligands, respectively. Aquation is commonly accepted as the mode of action whereby the malonato-amido ligands dissociates from the platinum center upon contact with aqueous solution. The rate of aquation is the key in determining drug reactivity and active drug circulation duration in the body. A comprehensive review detailing the development of PHPMA-anticancer conjugates was reported by Duncan recently [123].

Unlike PEG and PHPMA, PGA is a poly amino acid that is biodegradable. It is generally non-toxic and well-tolerated in the body. There are currently two PGA–drug conjugates in clinical trials, namely PGA–PTX (Xyotax/CT2103) [132,133] and PGA–CPT (CT2106) [134–136]. PTX is covalently bound to PGA through an ester linkage on the 2′-OH site in CT2103, while CPT is attached to PGA *via* an amide-bond on the 20S-OH position in CT2106. In the latter example, conjugation of CPT was shown to prevent ring opening of the lactone fragment, thereby enhancing its solubility and biodistribution. Both CT2103 and CT2106 contain enzymatically cleavable bonds that can result in eventual drug release from PGA. Alternatively, non-specific protease action and abiotic hydrolysis may degrade the PGA backbone, giving rise to oligo glutamyl–drug conjugates. Several hybrid PEG–(PGA–drug) prodrug systems have been developed by Kataoka and coworkers including PEG–(PGA–cisplatin) (NC-6004) [137], PEG–(PGA–DACHPt) (NC-4016) [1] and PEG–(PGA–SN38) (NK-012) [138]. In the case of platinum-containing chemotherapeutics, the metal was coordinated to the carboxylato-ligands tethered on the deprotonated glutamic acid; each platinum-center can be coordinated to two adjacent carboxylato-ligands on the same polymer backbone, or it can adopt a bridging configuration to another polymer strain of PGA. In NK-012, the drug molecule, SN38, was covalently attached to the PGA copolymer forming a phenyl–ester bond; this ester linkage can gradually be cleaved by hydrolysis under physiological conditions to release the active drug in a non-enzymatic manner.

Another micelle carrier system for DOX, PEG–(P(Asp)–DOX) (NK911), has also been described by Kataoka and coworkers [139,140]. Here, DOX was partially conjugated (ca. 45%) to the Asp side chain to confer greater hydrophobicity to the micellar inner core. Subsequently, additional free DOX was loaded into the micelles *via* physical interactions between the conjugated and free drug. The drug loading capacity, stability and release profile of NK911 can be controlled by the degree of DOX conjugation. The physically-loaded DOX can be released gradually between 8 and 24 h to exert the desired anti-tumor activity. This system is currently undergoing a clinical II trial for the treatment of metastatic pancreatic cancer [1].

Apart from the above-mentioned clinical examples, there are many other elegant polymer–drug conjugate systems reported in the literature [117,141,142]; many of them contain a non-biodegradable polymeric backbone while some contain degradable ones, such as polycarbonate (PCB), PLA and polyphosphoester (PPE). Recently, Ke et al. reported the use of PEG-*b*-PCB conjugated with DOX; the chemical conjugation occurs between a benzaldehyde moiety tethered on the PCB side-arm and the $-NH_2$ group on DOX [23] (Fig. 9A). The resultant PEG-*b*-(PCB-DOX) contains imino-bonds that are pH-sensitive, and this was postulated to enhance intracellular release. More importantly, these drug-conjugated micelles were more potent against DOX-resistant human breast cancer MCF-7/Adr cells compared to the free drug, and killed the cancer cells more effectively at the same drug concentration. In a separate report, Wooley and coworkers chemically linked PTX to PEO-*b*-PPE copolymers to achieve ultra-high drug-loaded multifunctional particles of up to 65 wt.% [143] (Fig. 9B). It was demonstrated that a maximum PTX concentration of 6.2 mg/mL in water was achieved, resulting in a 25,000-fold increase in comparison to the free drug. Here, PTX was chemically-altered onto an azido-platform using DCC coupling before conjugation to an alkyne-modified PEO-*b*-PPE using the popular azide-alkyne "click chemistry". PEO-*b*-(PPE–PTX) was shown to be potent against several cell lines including OVCAR-3 human ovarian carcinoma, RAW 264.7 mouse leukaemic monocyte macrophage, KB and A549 human lung cancer cell lines. In another recent example [144], Cheng and co-workers have brilliantly made use of the hydroxyl group on anticancer drugs, *e.g.* PTX, DOX and DTX, to initiate the ROP of phenyl-derivatized *O*-carboxyanhydrides (Phe-OCA) for the preparation of drug–$PheLA_n$ nanoconjugates in the presence of a β-diimine–Zn complex. CPT-$PheLA_n$ was subsequently nanoprecipitated with mPEG–$PheLA_{100}$ to generate the desired nanodrug vehicle (Fig. 9C). They have also employed a similar strategy using PLA to target other drug–PLA conjugate systems [145–147].

Curcumin is a hydrophobic molecule and exhibits poor dietary bioavailability. Despite these limitations, there are currently a number of clinical trials in humans studying the effect of curcumin as an anticancer agent. Chemical conjugation of curcumin to hydrophilic polymers has been employed to substantially improve its aqueous solubility [148–150]. In one report, curcumin was conjugated to mPEG–PLA using a tris(hydroxymethyl)aminomethane (Tris) linker *via* ester-bonds; the CMC values of mPEG–(PLA–Tris-curcumin) and mPEG–(PLA–curcumin) conjugates were found to be 10 times lower than that of mPEG–PLA, indicating an improved thermodynamic stability of micelles due to stronger hydrophobic interaction within the polymeric core [151]. Furthermore, curcumin loading in mPEG–(PLA–Tris–curcumin) micelles was found to be much higher than conventional mPEG–PLA micelles, reaching 18.5 ± 1.3 wt.% and 3.6 ± 0.4 wt.%, respectively. The ester-linkages can easily be cleaved enzymatically or through acid/base-catalyzed hydrolysis.

The field of polymer–drug conjugation chemistry is an active and fast-growing area both in the academic laboratories as well as in the pharmaceutical industry. Key advantages of polymer–drug conjugated systems include prolonged half-life, greater hydrophilicity and stability, lower immunogenicity and toxicity, as well as enhancement of targeting specificity. However, chemically-conjugated polymeric delivery vehicles often suffer from slow drug release due to the strong covalent interactions, which translate to low pharmacologically relevant drug concentrations. In order to facilitate controlled-release of the active drug at targeted sites, stimuli-triggering cleavage of polymer-drug linkages is critical; strategies include (1) pH-sensitive linkers, *e.g.* hydrazone (–C═N–NHR) and imine (–C═N–R) bonds [152], (2) biologically reductive-sensitive linkages, *e.g.* disulfide bonds (S–S) [153], and (3) enzymatically cleavable short peptide linkers [154]. Although there is still an obvious lag in translating these polymer–drug conjugates into actual clinical applications, there remain good opportunities in the pursuit of such research in the face of ever-growing healthcare demands.

7. Conclusion and future perspectives

In this review, we have summarized the elegant usage of both non-covalent and covalent interactions for the assembly of various micellar drug delivery systems. Specific polymer–drug interactions are critical in the preparation of micelles with effective physicochemical properties including enhanced drug stability and increased drug loading capacity. In general, the "like attracts like" principle for generating stable micellar core applies, for instance, hydrophobic core attracts hydrophobic drugs, and hydrogen-bond donor attracts hydrogen-bond acceptor. Through adjusting these interactions, polymeric architectures and compositions can be better-tuned to optimize micelles stability and drug loading efficacy. Additionally, multiple interactions can be constructed within a single micellar system for the co-delivery of multiple drugs so as to achieve sequential drug release or to accomplish synergistic therapeutic effects. Chemical cross-linking of micellar systems and polymer–drug chemical conjugation can also be exploited to further stabilize macromolecular therapeutics during systemic circulation; however, the robust covalent bonding may adversely affect the rate of cargo release, hence the use of stimulus-responsive interactions are preferred.

In order to design an efficient micellar system for specific drug delivery, advanced synthetic polymer chemistry is needed especially for the preparation of well-defined polymeric architecture and composition with predictable molecular weights and narrow polydispersity. The range of well-defined synthetic micellar polymeric systems is substantially boosted by the advent of well-controlled polymerization techniques including ATRP [155], reversible addition-fragmentation chain-transfer polymerization (RAFT) [156], ROMP [157], and ROP

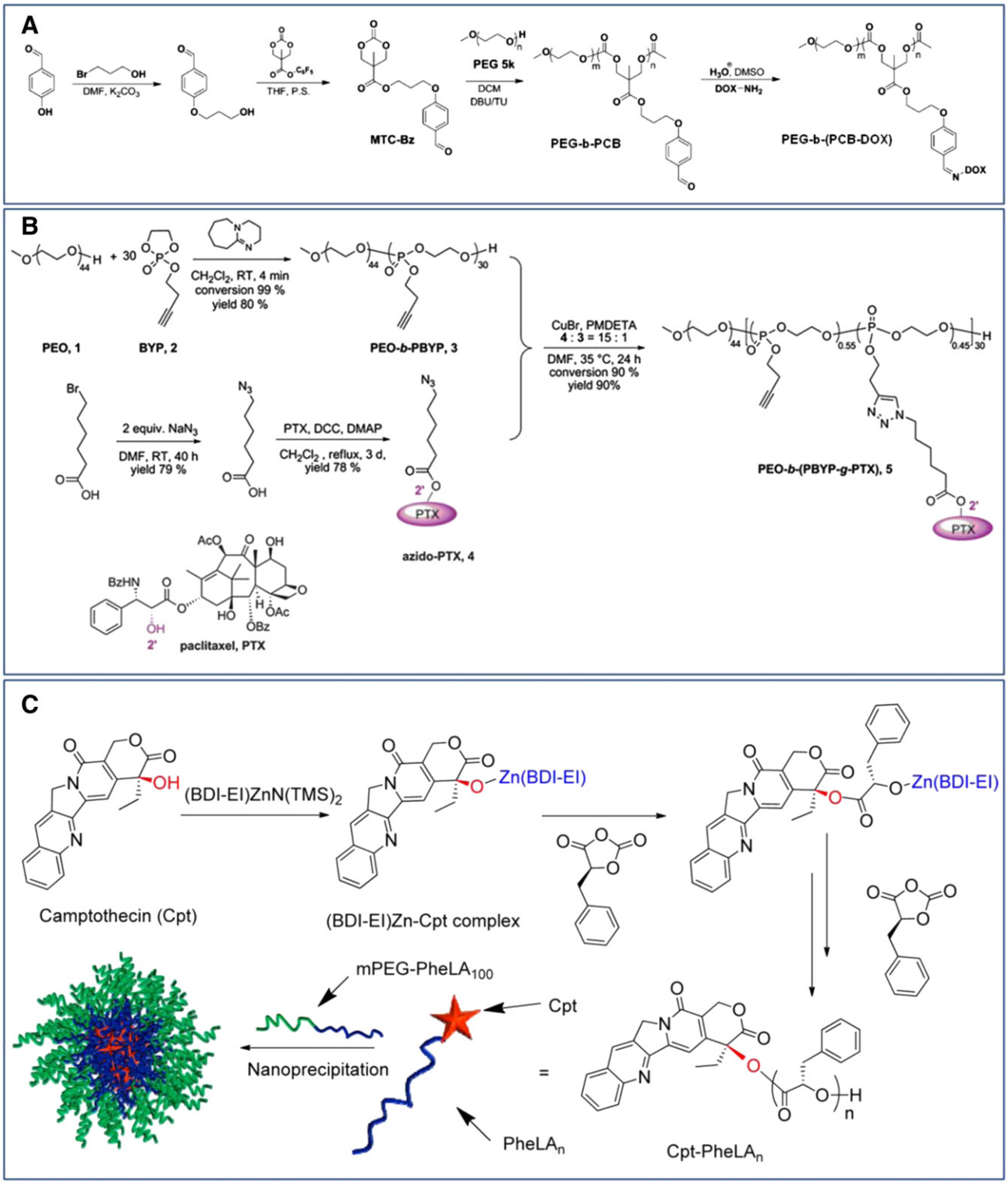

Fig. 9. Schematic representation of the synthesis of PEG-*b*-(PCB-DOX) (A), PEO-*b*-(PBYP-*g*-PTX) (B) and Cpt-PheLA$_n$ nanoconjugates (C). Reprinted with permission from references [23,143] and [144].

[158]. As the toolbox for the construction of such biomaterials expands, the flexibility and opportunity to better engineer drug delivery systems to suit specific applications will inevitably be enriched. Furthermore, one can have easy access to both biodegradable as well as non-biodegradable polymeric materials depending on the choice of the polymerization methodology. The popularity of polymerization techniques such as ATRP, RAFT and ROMP is predominantly enhanced by abundant low cost starting materials (*e.g.* derivatized (meth)acrylates and norbornenes), ease of polymerization control and monomer-catalyst compatibility; however, they generally give rise to materials that are non-biodegradable, and require the use of toxic metal catalysts. On the other hand, the development of well-defined biodegradable systems, such as polycarbonates, polyesters, polylactones, and polyphosphoesters, can easily be achieved from their respective cyclic precursors using a variety of organocatalysts; this eliminates the utilization of toxic metal catalysts, which is highly advantageous for

biomedical applications. Notably, tailor-made micellar drug delivery vehicles are expected to be made possible and accessible with the advancement of synthetic polymer chemistry.

The assembly of effective drug delivery systems impinges on optimal interactions between the polymer and the drug. In addition, better understanding of cancer type, tumor location and its microenvironment will also play a major role in determining the eventual treatment outcome [159]. The 'one-size-fits-all' approach must be re-evaluated and replaced with more personalized treatment in order for successful clinical application of these nanosized delivery systems. Although this concept may appear challenging, it can give rise to countless realistic opportunities and possibilities. And as the field of polymeric micellar drug delivery systems continue to mature, there is no doubt that targeted delivery and personalized therapy would take center-stage. With cooperation between scientists, clinicians, pharmaceutical industry and legislative bodies, more drug delivery systems would be applied in clinic for improved therapy.

Acknowledgments

This work was funded by the Institute of Bioengineering and Nanotechnology (Biomedical Research Council, Agency for Science, Technology and Research, Singapore), and IBM Almaden Research Center.

References

[1] J. Gong, M. Chen, Y. Zheng, S. Wang, Y. Wang, Polymeric micelles drug delivery system in oncology, J. Control. Release 159 (2012) 312–323.
[2] Z. Liu, Y. Wang, N. Zhang, Micelle-like nanoassemblies based on polymer–drug conjugates as an emerging platform for drug delivery, Expert Opin. Drug Deliv. 9 (2012) 805–822.
[3] M. Oba, Study on development of polymeric micellar gene carrier and evaluation of its functionality, Biol. Pharm. Bull. 36 (2013) 1045–1051.
[4] N. Wiradharma, Y. Zhang, S. Venkataraman, J.L. Hedrick, Y.Y. Yang, Self-assembled polymer nanostructures for delivery of anticancer therapeutics, Nano Today 4 (2009) 302–317.
[5] S. Kim, Y. Shi, J.Y. Kim, K. Park, J.X. Cheng, Overcoming the barriers in micellar drug delivery: loading efficiency, *in vivo* stability, and micelle–cell interaction, Expert Opin. Drug Deliv. 7 (2010) 49–62.
[6] A.B. Ebrahim Attia, Z.Y. Ong, J.L. Hedrick, P.P. Lee, P.L.R. Ee, P.T. Hammond, Y.-Y. Yang, Mixed micelles self-assembled from block copolymers for drug delivery, Curr. Opin. Colloid Interface Sci. 16 (2011) 182–194.
[7] H. Maeda, Vascular permeability in cancer and infection as related to macromolecular drug delivery, with emphasis on the EPR effect for tumor-selective drug targeting, Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 88 (2012) 53–71.
[8] V. Torchilin, Tumor delivery of macromolecular drugs based on the EPR effect, Adv. Drug Deliv. Rev. 63 (2011) 131–135.
[9] C.M. Hu, L. Zhang, Therapeutic nanoparticles to combat cancer drug resistance, Curr. Drug Metab. 10 (2009) 836–841.
[10] C. Allena, D. Maysingerb, A. Eisenberga, Nano-engineering block copolymer aggregates for drug delivery, Colloids Surf., B 6 (1999) 3–27.
[11] Y.H. Bae, H. Yin, Stability issues of polymeric micelles, J. Control. Release 131 (2008) 2–4.
[12] J. Liu, F. Zeng, C. Allen, *In vivo* fate of unimers and micelles of a poly(ethylene glycol)-block-poly(caprolactone) copolymer in mice following intravenous administration, Eur. J. Pharm. Biopharm. 65 (2007) 309–319.
[13] E.V. Batrakova, S. Li, Y. Li, V.Y. Alakhov, W.F. Elmquist, A.V. Kabanov, Distribution kinetics of a micelle-forming block copolymer Pluronic P85, J. Control. Release 100 (2004) 389–397.
[14] C. Allena, D. Maysiger, A. Eisenberg, Nano-engineering block copolymer aggregates for drug delivery, Colloids Surf., B 16 (1999) 3–27.
[15] K. Fukushima, R.C. Pratt, F. Nederberg, J.P. Tan, Y.Y. Yang, R.M. Waymouth, J.L. Hedrick, Organocatalytic approach to amphiphilic comb-block copolymers capable of stereocomplexation and self-assembly, Biomacromolecules 9 (2008) 3051–3056.
[16] A.L. Lee, S. Venkataraman, S.B. Sirat, S. Gao, J.L. Hedrick, Y.Y. Yang, The use of cholesterol-containing biodegradable block copolymers to exploit hydrophobic interactions for the delivery of anticancer drugs, Biomaterials 33 (2012) 1921–1928.
[17] J.P. Tan, S.H. Kim, F. Nederberg, K. Fukushima, D.J. Coady, A. Nelson, Y.Y. Yang, J.L. Hedrick, Delivery of anticancer drugs using polymeric micelles stabilized by hydrogen-bonding urea groups, Macromol. Rapid Commun. 31 (2010) 1187–1192.
[18] S.H. Kim, J.P. Tan, F. Nederberg, K. Fukushima, J. Colson, C. Yang, A. Nelson, Y.Y. Yang, J.L. Hedrick, Hydrogen bonding-enhanced micelle assemblies for drug delivery, Biomaterials 31 (2010) 8063–8071.
[19] C. Yang, J.P.K. Tan, W. Cheng, A.B.E. Attia, C.T.Y. Ting, A. Nelson, J.L. Hedrick, Y.-Y. Yang, Supramolecular nanostructures designed for high cargo loading capacity and kinetic stability, Nano Today 5 (2010) 515–523.
[20] C. Yang, A.B. Attia, J.P. Tan, X. Ke, S. Gao, J.L. Hedrick, Y.Y. Yang, The role of non-covalent interactions in anticancer drug loading and kinetic stability of polymeric micelles, Biomaterials 33 (2012) 2971–2979.
[21] A.B. Attia, C. Yang, J.P. Tan, S. Gao, D.F. Williams, J.L. Hedrick, Y.Y. Yang, The effect of kinetic stability on biodistribution and anti-tumor efficacy of drug-loaded biodegradable polymeric micelles, Biomaterials 34 (2013) 3132–3140.
[22] X.Y. Ke, V.W. Lin Ng, S.J. Gao, Y.W. Tong, J.L. Hedrick, Y.Y. Yang, Co-delivery of thioridazine and doxorubicin using polymeric micelles for targeting both cancer cells and cancer stem cells, Biomaterials 35 (2014) 1096–1108.
[23] X. Ke, D.J. Coady, C. Yang, A.C. Engler, J.L. Hedrick, Y.Y. Yang, pH-sensitive polycarbonate micelles for enhanced intracellular release of anticancer drugs: a strategy to circumvent multidrug resistance, Polym. Chem. 5 (2014) 2621.
[24] J.Z. Du, X.J. Du, C.Q. Mao, J. Wang, Tailor-made dual pH-sensitive polymer-doxorubicin nanoparticles for efficient anticancer drug delivery, J. Am. Chem. Soc. 133 (2011) 17560–17563.
[25] C. Chang, H. Wei, J. Feng, Z.-C. Wang, X.-J. Wu, D.-Q. Wu, S.-X. Cheng, X.-Z. Zhang, R.-X. Zhuo, Temperature and pH double responsive hybrid cross-linked micelles based on P(NIPAAm-co-MPMA)-b-P(DEA): RAFT synthesis and "schizophrenic" micellization, Macromolecules 42 (2009) 4838–4844.
[26] S.Q. Liu, N. Wiradharma, S.J. Gao, Y.W. Tong, Y.Y. Yang, Bio-functional micelles self-assembled from a folate-conjugated block copolymer for targeted intracellular delivery of anticancer drugs, Biomaterials 28 (2007) 1423–1433.
[27] W.Y. Seow, J.M. Xue, Y.Y. Yang, Targeted and intracellular delivery of paclitaxel using multi-functional polymeric micelles, Biomaterials 28 (2007) 1730–1740.
[28] V.A. Sethuraman, Y.H. Bae, TAT peptide-based micelle system for potential active targeting of anti-cancer agents to acidic solid tumors, J. Control. Release 118 (2007) 216–224.
[29] Z.G. Gao, L. Tian, J. Hu, I.S. Park, Y.H. Bae, Prevention of metastasis in a 4T1 murine breast cancer model by doxorubicin carried by folate conjugated pH sensitive polymeric micelles, J. Control. Release 152 (2011) 84–89.
[30] O.C. Farokhzad, J. Cheng, B.A. Teply, I. Sherifi, S. Jon, P.W. Kantoff, J.P. Richie, R. Langer, Targeted nanoparticle–aptamer bioconjugates for cancer chemotherapy *in vivo*, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 6315–6320.
[31] S.Y. Lee, S. Kim, J.Y. Tyler, K. Park, J.X. Cheng, Blood-stable, tumor-adaptable disulfide bonded mPEG–(Cys)4–PDLLA micelles for chemotherapy, Biomaterials 34 (2013) 552–561.
[32] H. Cabral, N. Nishiyama, S. Okazaki, H. Koyama, K. Kataoka, Preparation and biological properties of dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt)-loaded polymeric micelles, J. Control. Release 101 (2005) 223–232.
[33] C.V. Synatschke, T. Nomoto, H. Cabral, M. Fortsch, K. Toh, Y. Matsumoto, K. Miyazaki, A. Hanisch, F.H. Schacher, A. Kishimura, N. Nishiyama, A.H. Muller, K. Kataoka, Multicompartment micelles with adjustable poly(ethylene glycol) shell for efficient *in vivo* photodynamic therapy, ACS Nano 8 (2014) 1161–1172.
[34] D. Kim, Z.G. Gao, E.S. Lee, Y.H. Bae, *In vivo* evaluation of doxorubicin-loaded polymeric micelles targeting folate receptors and early endosomal pH in drug-resistant ovarian cancer, Mol. Pharm. 6 (2009) 1353–1362.
[35] M.P. Xiong, J.A. Yanez, C.M. Remsberg, Y. Ohgami, G.S. Kwon, N.M. Davies, M.L. Forrest, Formulation of a geldanamycin prodrug in mPEG-b-PCL micelles greatly enhances tolerability and pharmacokinetics in rats, J. Control. Release 129 (2008) 33–40.
[36] D.W. Kim, S.Y. Kim, H.K. Kim, S.W. Kim, S.W. Shin, J.S. Kim, K. Park, M.Y. Lee, D.S. Heo, Multicenter phase II trial of Genexol-PM, a novel Cremophor-free, polymeric micelle formulation of paclitaxel, with cisplatin in patients with advanced non-small-cell lung cancer, Ann. Oncol. 18 (2007) 2009–2014.
[37] K.S. Lee, H.C. Chung, S.A. Im, Y.H. Park, C.S. Kim, S.B. Kim, S.Y. Rha, M.Y. Lee, J. Ro, Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer, Breast Cancer Res. Treat. 108 (2008) 241–250.
[38] T. Hamaguchi, Y. Matsumura, M. Suzuki, K. Shimizu, R. Goda, I. Nakamura, I. Nakatomi, M. Yokoyama, K. Kataoka, T. Kakizoe, NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend *in vivo* antitumour activity and reduce the neurotoxicity of paclitaxel, Br. J. Cancer 92 (2005) 1240–1246.
[39] K. Kato, K. Chin, T. Yoshikawa, K. Yamaguchi, Y. Tsuji, T. Esaki, K. Sakai, M. Kimura, T. Hamaguchi, Y. Shimada, Y. Matsumura, R. Ikeda, Phase II study of NK105, a paclitaxel-incorporating micellar nanoparticle, for previously treated advanced or recurrent gastric cancer, Invest. New Drugs 30 (2012) 1621–1627.
[40] J. Varshosaz, F. Hasanzadeh, M. Eslamdoost, Optimization of self-assembling properties of fatty acids grafted to methoxy poly(ethylene glycol) as nanocarriers for etoposide, Acta Pharm. 62 (2012) 31–44.
[41] S. Alexander, T. Cosgrove, S.W. Prescott, T.C. Castle, Flurbiprofen encapsulation using pluronic triblock copolymers, Langmuir 27 (2011) 8054–8060.
[42] D. Guenard, F. Gueritte-Voegelein, P. Potier, Taxol and taxotere: discovery, chemistry, and structure–activity relationships, Acc. Chem. Res. 26 (1993) 160–167.
[43] J.P. Tan, S.H. Kim, F. Nederberg, E.A. Appel, R.M. Waymouth, Y. Zhang, J.L. Hedrick, Y.Y. Yang, Hierarchical supermolecular structures for sustained drug release, Small 5 (2009) 1504–1507.
[44] X.D. Guo, J.P. Tan, S.H. Kim, L.J. Zhang, Y. Zhang, J.L. Hedrick, Y.Y. Yang, Y. Qian, Computational studies on self-assembled paclitaxel structures: templates for hierarchical block copolymer assemblies and sustained drug release, Biomaterials 30 (2009) 6556–6563.
[45] M.G. Carstens, P.H. de Jong, C.F. van Nostrum, J. Kemmink, R. Verrijk, L.G. de Leede, D.J. Crommelin, W.E. Hennink, The effect of core composition in biodegradable oligomeric micelles as taxane formulations, Eur. J. Pharm. Biopharm. 68 (2008) 596–606.

[46] Y. Wang, S. Gao, W.H. Ye, H.S. Yoon, Y.Y. Yang, Co-delivery of drugs and DNA from cationic core–shell nanoparticles self-assembled from a biodegradable copolymer, Nat. Mater. 5 (2006) 791–796.
[47] A.L. Lee, Y. Wang, H.Y. Cheng, S. Pervaiz, Y.Y. Yang, The co-delivery of paclitaxel and Herceptin using cationic micellar nanoparticles, Biomaterials 30 (2009) 919–927.
[48] A.L. Lee, Y. Wang, S. Pervaiz, W. Fan, Y.Y. Yang, Synergistic anticancer effects achieved by co-delivery of TRAIL and paclitaxel using cationic polymeric micelles, Macromol. Biosci. 11 (2011) 296–307.
[49] T. Jiang, Y. Li, Y. Lv, Y. Cheng, F. He, R. Zhuo, Biodegradable amphiphilic block-graft copolymers based on methoxy poly(ethylene glycol)-b-(polycarbonates-g-polycarbonates) for controlled release of doxorubicin, J. Mater. Sci. Mater. Med. 25 (2014) 131–139.
[50] Y. Sun, W. Zou, S. Bian, Y. Huang, Y. Tan, J. Liang, Y. Fan, X. Zhang, Bioreducible PAA-g-PEG graft micelles with high doxorubicin loading for targeted antitumor effect against mouse breast carcinoma, Biomaterials 34 (2013) 6818–6828.
[51] Y.Z. Du, Q. Weng, H. Yuan, F.Q. Hu, Synthesis and antitumor activity of stearate-g-dextran micelles for intracellular doxorubicin delivery, ACS Nano 4 (2010) 6894–6902.
[52] V.P. Torchilin, Structure and design of polymeric surfactant-based drug delivery systems, J. Control. Release 73 (2001) 137–172.
[53] X. Chang, C.M. Dong, Synthesis of hyperbranched polypeptide and PEO block copolymer by consecutive thiol-yne chemistry, Biomacromolecules 14 (2013) 3329–3337.
[54] C. Li, B. Wang, Y. Liu, J. Cao, T. Feng, Y. Chen, X. Luo, Synthesis and evaluation of star-shaped poly(-caprolactone)–poly(2-hydroxyethyl methacrylate) as potential anticancer drug delivery carriers, J. Biomater. Sci. Polym. Ed. 24 (2013) 741–757.
[55] Z. Poon, S. Chen, A.C. Engler, H.I. Lee, E. Atas, G. von Maltzahn, S.N. Bhatia, P.T. Hammond, Ligand-clustered "patchy" nanoparticles for modulated cellular uptake and *in vivo* tumor targeting, Angew. Chem. Int. Ed. Engl. 49 (2010) 7266–7270.
[56] Z. Poon, J.A. Lee, S. Huang, R.J. Prevost, P.T. Hammond, Highly stable, ligand-clustered "patchy" micelle nanocarriers for systemic tumor targeting, Nanomedicine 7 (2011) 201–209.
[57] Y. Ikada, K. Jamshidi, H. Tsuji, S.H. Hyon, Stereocomplex formation between enantiomeric poly(lactides), Macromolecules 20 (1987) 904–906.
[58] J. Zhang, K. Tashiro, H. Tsuji, A.J. Domb, Investigation of phase transitional behavior of poly(L-lactide)/poly(D-lactide) blend used to prepare the highly-oriented stereocomplex, Macromolecules 40 (2007) 1049–1054.
[59] J.R. Sarasua, N.L. Rodríguez, A.L. Arraiza, E. Meaurio, Stereoselective crystallization and specific interactions in polylactides, Macromolecules 38 (2005) 8362–8371.
[60] N. Kang, M.E. Perron, R.E. Prud'homme, Y. Zhang, G. Gaucher, J.C. Leroux, Stereocomplex block copolymer micelles: core–shell nanostructures with enhanced stability, Nano Lett. 5 (2005) 315–319.
[61] S.H. Kim, J.P.K. Tan, F. Nederberg, K. Fukushima, Y.Y. Yang, R.M. Waymouth, J.L. Hedrick, Mixed micelle formation through stereocomplexation between enantiomeric poly (lactide) block copolymers, Macromolecules 42 (2009) 25–29.
[62] R.J. Pounder, H. Willcock, N.S. Ieong, R.K. O'Reilly, A.P. Dove, Stereocomplexation in novel degradable amphiphilic block copolymer micelles of poly(ethylene oxide) and poly(benzyl α-malate), Soft Matter 7 (2011) 10987.
[63] S.W. Tan, H.J. Wang, K.H. Tu, H.L. Jiang, L.Q. Wang, Ibuprofen induced drug loaded polymeric micelles, Chin. Chem. Lett. 22 (2011) 1123–1126.
[64] L.-Q. Wang, K. Tu, Y. Li, J. Zhang, L. Jiang, Z. Zhang, Synthesis and characterization of temperature responsive graft copolymers of dextran with poly(N-isopropylacrylamide), React. Funct. Polym. 53 (2002) 19–27.
[65] J.X. Zhang, M.Q. Yan, X.H. Li, L.Y. Qiu, X.D. Li, X.J. Li, Y. Jin, K.J. Zhu, Local delivery of indomethacin to arthritis-bearing rats through polymeric micelles based on amphiphilic polyphosphazenes, Pharm. Res. 24 (2007) 1944–1953.
[66] W. Su, X.H. Luo, H.F. Wang, L. Li, J. Feng, X.Z. Zhang, R.X. Zhuo, Hyperbranched polycarbonate-based multimolecular micelle with enhanced stability and loading efficiency, Macromol. Rapid Commun. 32 (2011) 390–396.
[67] H. Kuang, S. Wu, Z. Xie, F. Meng, X. Jing, Y. Huang, Biodegradable amphiphilic copolymer containing nucleobase: synthesis, self-assembly in aqueous solutions, and potential use in controlled drug delivery, Biomacromolecules 13 (2012) 3004–3012.
[68] A. Ruiz de Luzuriaga, I. García, D. Mecerreyes, A. Etxeberria, J.A. Pomposo, Design and stabilization of block copolymer micelles via phenol–pyridine hydrogen-bonding interactions, Polymer 51 (2010) 1355–1362.
[69] J.X. Zhang, X.J. Li, L.Y. Qiu, X.H. Li, M.Q. Yan, J. Yi, K.J. Zhu, Indomethacin-loaded polymeric nanocarriers based on amphiphilic polyphosphazenes with poly (N-isopropylacrylamide) and ethyl tryptophan as side groups: Preparation, *in vitro* and *in vivo* evaluation, J. Control. Release 116 (2006) 322–329.
[70] Y.I. Jeong, S.H. Kim, T.Y. Jung, I.Y. Kim, S.S. Kang, Y.H. Jin, H.H. Ryu, H.S. Sun, S. Jin, K. K. Kim, K.Y. Ahn, S. Jung, Polyion complex micelles composed of all-trans retinoic acid and poly (ethylene glycol)-grafted-chitosan, J. Pharm. Sci. 95 (2006) 2348–2360.
[71] K.W. Yang, X.R. Li, Z.L. Yang, P.Z. Li, F. Wang, Y. Liu, Novel polyion complex micelles for liver-targeted delivery of diammonium glycyrrhizinate: *in vitro* and *in vivo* characterization, J. Biomed. Mater. Res. Part A 88 (2009) 140–148.
[72] E. He, C.Y. Yue, K.C. Tam, Binding and release studies of a cationic drug from a star-shaped four-arm poly(ethylene oxide)-b-poly(methacrylic acid), J. Pharm. Sci. 99 (2010) 782–793.
[73] A.M. Eckman, E. Tsakalozou, N.Y. Kang, A. Ponta, Y. Bae, Drug release patterns and cytotoxicity of PEG–poly(aspartate) block copolymer micelles in cancer cells, Pharm. Res. 29 (2012) 1755–1767.
[74] A.L. Lee, Y. Wang, W.H. Ye, H.S. Yoon, S.Y. Chan, Y.Y. Yang, Efficient intracellular delivery of functional proteins using cationic polymer core/shell nanoparticles, Biomaterials 29 (2008) 1224–1232.
[75] A. Harada, K. Kataoka, Novel polyion complex micelles entrapping enzyme molecules in the core: preparation of narrowly-distributed micelles from lysozyme and poly(ethylene glycol)–poly(aspartic acid) block copolymer in aqueous medium, Macromolecules 31 (1998) 288–294.
[76] F. Wang, X. Li, Y. Zhou, Y. Zhang, X. Chen, J. Yang, Y. Huang, Y. Liu, Nanoscaled polyion complex micelles for targeted delivery of recombinant hirudin to platelets based on cationic copolymer, Mol. Pharm. 7 (2010) 718–726.
[77] Y. Lee, S. Fukushima, Y. Bae, S. Hiki, T. Ishii, K. Kataoka, A protein nanocarrier from charge-conversion polymer in response to endosomal pH, J. Am. Chem. Soc. 129 (2007) 5362–5363.
[78] Y. Lee, T. Ishii, H.J. Kim, N. Nishiyama, Y. Hayakawa, K. Itaka, K. Kataoka, Efficient delivery of bioactive antibodies into the cytoplasm of living cells by charge-conversional polyion complex micelles, Angew. Chem. Int. Ed. Engl. 49 (2010) 2552–2555.
[79] K. Kataoka, H. Togawa, A. Harada, K. Yasugi, T. Matsumoto, S. Katayose, Spontaneous formation of polyion complex micelles with narrow distribution from antisense oligonucleotide and cationic block copolymer in physiological saline, Macromolecules 29 (1996) 8556–8557.
[80] Y. Wang, L.S. Wang, S.H. Goh, Y.Y. Yang, Synthesis and characterization of cationic micelles self-assembled from a biodegradable copolymer for gene delivery, Biomacromolecules 8 (2007) 1028–1037.
[81] C.W. Beh, W.Y. Seow, Y. Wang, Y. Zhang, Z.Y. Ong, P.L. Ee, Y.Y. Yang, Efficient delivery of Bcl-2-targeted siRNA using cationic polymer nanoparticles: downregulating mRNA expression level and sensitizing cancer cells to anticancer drug, Biomacromolecules 10 (2009) 41–48.
[82] N. Wiradharma, M. Khan, Y.W. Tong, S. Wang, Y.-Y. Yang, Self-assembled cationic peptide nanoparticles capable of inducing efficient gene expression *in vitro*, Adv. Funct. Mater. 18 (2008) 943–951.
[83] X.D. Guo, F. Tandiono, N. Wiradharma, D. Khor, C.G. Tan, M. Khan, Y. Qian, Y.Y. Yang, Cationic micelles self-assembled from cholesterol-conjugated oligopeptides as an efficient gene delivery vector, Biomaterials 29 (2008) 4838–4846.
[84] P. Chan, M. Kurisawa, J.E. Chung, Y.Y. Yang, Synthesis and characterization of chitosan-g-poly(ethylene glycol)-folate as a non-viral carrier for tumor-targeted gene delivery, Biomaterials 28 (2007) 540–549.
[85] R.J. Christie, Y. Matsumoto, K. Miyata, T. Nomoto, S. Fukushima, K. Osada, J. Halnaut, F. Pittella, H.J. Kim, N. Nishiyama, K. Kataoka, Targeted polymeric micelles for siRNA treatment of experimental cancer by intravenous injection, ACS Nano 6 (2012) 5174–5189.
[86] Y. Qian, Y. Zha, B. Feng, Z. Pang, B. Zhang, X. Sun, J. Ren, C. Zhang, X. Shao, Q. Zhang, X. Jiang, PEGylated poly(2-(dimethylamino) ethyl methacrylate)/DNA polyplex micelles decorated with phage-displayed TGN peptide for brain-targeted gene delivery, Biomaterials 34 (2013) 2117–2129.
[87] M. Harada-Shiba, K. Yamauchi, A. Harada, I. Takamisawa, K. Shimokado, K. Kataoka, Polyion complex micelles as vectors in gene therapy—pharmacokinetics and *in vivo* gene transfer, Gene Ther. 9 (2002) 407–414.
[88] X.J. Loh, S.J. Ong, Y.T. Tung, H.T. Choo, Co-delivery of drug and DNA from cationic dual-responsive micelles derived from poly(DMAEMA-co-PPGMA), Mater. Sci. Eng. C Mater. Biol. Appl. 33 (2013) 4545–4550.
[89] W. Kim, Y. Yamasaki, W.D. Jang, K. Kataoka, Thermodynamics of DNA condensation induced by poly(ethylene glycol)-block-polylysine through polyion complex micelle formation, Biomacromolecules 11 (2010) 1180–1186.
[90] Y. Shao, W. Huang, C. Shi, S.T. Atkinson, J. Luo, Reversibly crosslinked nanocarriers for on-demand drug delivery in cancer treatment, Ther. Deliv. 3 (2012) 1409–1427.
[91] R.K. O'Reilly, C.J. Hawker, K.L. Wooley, Cross-linked block copolymer micelles: functional nanostructures of great potential and versatility, Chem. Soc. Rev. 35 (2006) 1068–1083.
[92] X. Shuai, T. Merdan, A.K. Schaper, F. Xi, T. Kissel, Core-cross-linked polymeric micelles as paclitaxel carriers, Bioconjug. Chem. 15 (2004) 441–448.
[93] H.S. Oberoi, F.C. Laquer, L.A. Marky, A.V. Kabanov, T.K. Bronich, Core cross-linked block ionomer micelles as pH-responsive carriers for cis-diamminedichloroplatinum(II), J. Control. Release 153 (2011) 64–72.
[94] D. Scott, J. Rohr, Y. Bae, Nanoparticulate formulations of mithramycin analogs for enhanced cytotoxicity, Int. J. Nanomedicine 6 (2011) 2757–2767.
[95] Y. Wu, W. Chen, F. Meng, Z. Wang, R. Cheng, C. Deng, H. Liu, Z. Zhong, Core-crosslinked pH-sensitive degradable micelles: a promising approach to resolve the extracellular stability versus intracellular drug release dilemma, J. Control. Release 164 (2012) 338–345.
[96] W. Lin, D. Kim, pH-sensitive micelles with cross-linked cores formed from polyaspartamide derivatives for drug delivery, Langmuir 27 (2011) 12090–12097.
[97] A.N. Koo, H.J. Lee, S.E. Kim, J.H. Chang, C. Park, C. Kim, J.H. Park, S.C. Lee, Disulfide-cross-linked PEG–poly(amino acid)s copolymer micelles for glutathione-mediated intracellular drug delivery, Chem. Commun. (Camb.) (2008) 6570–6572.
[98] R. Wei, L. Cheng, M. Zheng, R. Cheng, F. Meng, C. Deng, Z. Zhong, Reduction-responsive disassemblable core-cross-linked micelles based on poly(ethylene glycol)-b-poly(N-2-hydroxypropyl methacrylamide)-lipoic acid conjugates for triggered intracellular anticancer drug release, Biomacromolecules 13 (2012) 2429–2438.
[99] Y. Li, K. Xiao, J. Luo, W. Xiao, J.S. Lee, A.M. Gonik, J. Kato, T.A. Dong, K.S. Lam, Well-defined, reversible disulfide cross-linked micelles for on-demand paclitaxel delivery, Biomaterials 32 (2011) 6633–6645.
[100] J. Kato, Y. Li, K. Xiao, J.S. Lee, J. Luo, J.M. Tuscano, R.T. O'Donnell, K.S. Lam, Disulfide cross-linked micelles for the targeted delivery of vincristine to B-cell lymphoma, Mol. Pharm. 9 (2012) 1727–1735.
[101] L. Yan, W. Wu, W. Zhao, R. Qi, D. Cui, Z. Xie, Y. Huang, T. Tong, X. Jing, Reduction-sensitive core-cross-linked mPEG–poly(ester-carbonate) micelles for glutathione-triggered intracellular drug release, Polym. Chem. 3 (2012) 2403–2412.

[102] S. Bontha, A.V. Kabanov, T.K. Bronich, Polymer micelles with cross-linked ionic cores for delivery of anticancer drugs, J. Control. Release 114 (2006) 163–174.
[103] J.O. Kim, A.V. Kabanov, T.K. Bronich, Polymer micelles with cross-linked polyanion core for delivery of a cationic drug doxorubicin, J. Control. Release 138 (2009) 197–204.
[104] J.O. Kim, N.V. Nukolova, H.S. Oberoi, A.V. Kabanov, T.K. Bronich, Block ionomer complex micelles with cross-linked cores for drug delivery, Polym. Sci. Ser. A Chem. Phys. 51 (2009) 708–718.
[105] J.O. Kim, G. Sahay, A.V. Kabanov, T.K. Bronich, Polymeric micelles with ionic cores containing biodegradable cross-links for delivery of chemotherapeutic agents, Biomacromolecules 11 (2010) 919–926.
[106] M. Oba, K. Aoyagi, K. Miyata, Y. Matsumoto, K. Itaka, N. Nishiyama, Y. Yamasaki, H. Koyama, K. Kataoka, Polyplex micelles with cyclic RGD peptide ligands and disulfide cross-links directing to the enhanced transfection via controlled intracellular trafficking, Mol. Pharm. 5 (2008) 1080–1092.
[107] S. Matsumoto, R.J. Christie, N. Nishiyama, K. Miyata, A. Ishii, M. Oba, H. Koyama, Y. Yamasaki, K. Kataoka, Environment-responsive block copolymer micelles with a disulfide cross-linked core for enhanced siRNA delivery, Biomacromolecules 10 (2009) 119–127.
[108] Y. Kim, M.H. Pourgholami, D.L. Morris, M.H. Stenzel, Effect of cross-linking on the performance of micelles as drug delivery carriers: a cell uptake study, Biomacromolecules 13 (2012) 814–825.
[109] H. Wei, C. Cheng, C. Chang, W.Q. Chen, S.X. Cheng, X.Z. Zhang, R.X. Zhuo, Synthesis and applications of shell cross-linked thermoresponsive hybrid micelles based on poly(N-isopropylacrylamide-co-3-(trimethoxysilyl)propyl methacrylate)-b-poly(methyl methacrylate), Langmuir 24 (2008) 4564–4570.
[110] A. Li, H.P. Luehmann, G. Sun, S. Samarajeewa, J. Zou, S. Zhang, F. Zhang, M.J. Welch, Y. Liu, K.L. Wooley, Synthesis and *in vivo* pharmacokinetic evaluation of degradable shell cross-linked polymer nanoparticles with poly(carboxybetaine) versus poly(ethylene glycol) surface-grafted coatings, ACS Nano 6 (2012) 8970–8982.
[111] S. Samarajeewa, R. Shrestha, M. Elsabahy, A. Karwa, A. Li, R.P. Zentay, J.G. Kostelc, R. B. Dorshow, K.L. Wooley, *In vitro* efficacy of paclitaxel-loaded dual-responsive shell cross-linked polymer nanoparticles having orthogonally degradable disulfide cross-linked corona and polyester core domains, Mol. Pharm. 10 (2013) 1092–1099.
[112] P.N. Shah, L.Y. Lin, J.A. Smolen, J.A. Tagaev, S.P. Gunsten, D.S. Han, G.S. Heo, Y. Li, F. Zhang, S. Zhang, B.D. Wright, M.J. Panzner, W.J. Youngs, S.L. Brody, K.L. Wooley, C.L. Cannon, Synthesis, characterization, and *in vivo* efficacy of shell cross-linked nanoparticle formulations carrying silver antimicrobials as aerosolized therapeutics, ACS Nano 7 (2013) 4977–4987.
[113] M. Elsabahy, S. Zhang, F. Zhang, Z.J. Deng, Y.H. Lim, H. Wang, P. Parsamian, P.T. Hammond, K.L. Wooley, Surface charges and shell crosslinks each play significant roles in mediating degradation, biofouling, cytotoxicity and immunotoxicity for polyphosphoester-based nanoparticles, Sci. Rep. 3 (2013) 3313.
[114] V. Butun, X.S. Wang, M.V. de Paz Banez, K.L. Robinson, N.C. Billingham, S.P. Armes, Z. Tuzar, Synthesis of shell cross-linked micelles at high solids in aqueous media, Macromolecules 33 (2000) 1–3.
[115] S.S. Desale, S.M. Cohen, Y. Zhao, A.V. Kabanov, T.K. Bronich, Biodegradable hybrid polymer micelles for combination drug therapy in ovarian cancer, J. Control. Release 171 (2013) 339–348.
[116] X. Hu, X. Jing, Biodegradable amphiphilic polymer–drug conjugate micelles, Expert Opin. Drug Deliv. 6 (2009) 1079–1090.
[117] G. Pasut, F.M. Veronese, Polymer–drug conjugation, recent achievements and general strategies, Prog. Polym. Sci. 32 (2007) 933–961.
[118] G. Pasut, F.M. Veronese, PEG conjugates in clinical development or use as anticancer agents: an overview, Adv. Drug Deliv. Rev. 61 (2009) 1177–1188.
[119] H. Zhao, B. Rubio, P. Sapra, D. Wu, P. Reddy, P. Sai, A. Martinez, Y. Gao, Y. Lozanguiez, C. Longley, L.M. Greenberger, I.D. Horak, Novel prodrugs of SN38 using multiarm poly(ethylene glycol) linkers, Bioconjug. Chem. 19 (2008) 849–859.
[120] P. Sapra, H. Zhao, M. Mehlig, J. Malaby, P. Kraft, C. Longley, L.M. Greenberger, I.D. Horak, Novel delivery of SN38 markedly inhibits tumor growth in xenografts, including a camptothecin-11—refractory model, Clin. Cancer Res. 14 (2008) 1888–1896.
[121] C. Liu, F. Liu, L. Feng, M. Li, J. Zhang, N. Zhang, The targeted co-delivery of DNA and doxorubicin to tumor cells via multifunctional PEI–PEG based nanoparticles, Biomaterials 34 (2013) 2547–2564.
[122] P.A. Vasey, S.B. Kaye, R. Morrison, C. Twelves, P. Wilson, R. Duncan, A.H. Thomson, L.S. Murray, T.E. Hilditch, T. Murray, S. Burtles, D. Fraier, E. Frigerio, J. Cassidy, Phase I clinical and pharmacokinetic study of PK1 [N-(2-Hydroxypropyl)methacrylamide copolymer doxorubicin]: first member of a new class of chemotherapeutic agents—drug–polymer conjugates, Clin. Cancer Res. 5 (1999) 83–94.
[123] R. Duncan, Development of HPMA copolymer–anticancer conjugates: clinical experience and lessons learnt, Adv. Drug Deliv. Rev. 61 (2009) 1131–1148.
[124] B. Rihova, M. Bilej, V. Vetvicka, K. Ulbrich, J. Strohalm, J. Kopecek, R. Duncan, Biocompatibility of N-(2-hydroxypropyl) methacrylamide copolymers containing adriamycin: immunogenicity, and effect on haematopoietic stem cells in bone marrow *in vivo* and mouse splenocytes and human peripheral blood lymphocytes *in vitro*, Biomaterials 10 (1989) 335–342.
[125] A. Paul, M.J. Vicent, R. Duncan, Using small-angle neutron scattering to study the solution conformation of N-(2-hydroxypropyl)methacrylamide copolymer–doxorubicin conjugates, Biomacromolecules 8 (2007) 1573–1579.
[126] L.W. Seymour, D.R. Ferry, D. Anderson, S. Hesslewood, P.J. Julyan, R. Poyner, J. Doran, A.M. Young, S. Burtles, D.J. Kerr, L.W. Seymour, D.R. Ferry, D. Anderson, S. Hesslewood, P.J. Julyan, R. Poyner, J. Doran, A.M. Young, S. Burtles, D.J. Kerr, f.t.C. R.C.P.I.I.C.T. Committee, Hepatic drug targeting: phase I evaluation of polymer-bound doxorubicin, J. Clin. Oncol 20 (2002) 1668–1676.
[127] P. Sood, K.B. Thurmond, J.E. Jacob, L.K. Waller, G.O. Silva, D.R. Stewart, D.P. Nowotnik, Synthesis and characterization of AP5346, a novel polymer-linked diaminocyclohexyl platinum chemotherapeutic agent, Bioconjug. Chem. 17 (2006) 1270–1279.
[128] J.R. Rice, J.L. Gerberich, D.P. Nowotnik, S.B. Howell, Preclinical efficacy and pharmacokinetics of AP5346, a novel diaminocyclohexane-platinum tumor-targeting drug delivery system, Clin. Cancer Res. 12 (2006) 2248–2254.
[129] M. Campone, J. Rademaker-Lakhai, J. Bennouna, S. Howell, D. Nowotnik, J. Beijnen, J.M. Schellens, Phase I and pharmacokinetic trial of AP5346, a DACH–platinum–polymer conjugate, administered weekly for three out of every 4 weeks to advanced solid tumor patients, Cancer Chemother. Pharmacol. 60 (2007) 523–533.
[130] E. Gianasi, R.G. Buckley, J. Latigo, M. Wasil, R. Duncan, HPMA copolymers platinates containing dicarboxylato ligands. preparation, characterisation and *in vitro* and *in vivo* evaluation, J. Drug Target. 10 (2002) 549–556.
[131] J.M. Rademaker-Lakhai, C. Terret, S.B. Howell, C.M. Baud, R.F. de Boer, D. Pluim, J.H. Beijnen, J.H.M. Schellens, J.-P. Droz, A phase I and pharmacological study of the platinum polymer AP5280 given as an intravenous infusion once every 3 weeks in patients with solid tumors, Clin. Cancer Res. 10 (2004) 3386–3395.
[132] C.J. Langer, CT-2103: a novel macromolecular taxane with potential advantages compared with conventional taxanes, Clin. Lung Cancer 6 (2004) S85–S88.
[133] J. Singer, B. Baker, P. Vries, A. Kumar, S. Shaffer, E. Vawter, M. Bolton, P. Garzone, Poly-(L)-glutamic acid-paclitaxel (CT-2103) [XYOTAX], a biodegradable polymeric drug conjugate: characterization, preclinical pharmacology, and preliminary clinical data, Adv. Exp. Med. Biol. 519 (2003) 81–99.
[134] J.W. Singer, P. De Vries, R. Bhatt, J. Tulinsky, P. Klein, C. Li, L. Milas, R.A. Lewis, S. Wallace, Conjugation of camptothecins to poly-(L-glutamic acid), Ann. N. Y. Acad. Sci. 922 (2000) 136–150.
[135] J.W. Singer, R. Bhatt, J. Tulinsky, K.R. Buhler, E. Heasley, P. Klein, P. de Vries, Water-soluble poly-(L-glutamic acid)–Gly-camptothecin conjugates enhance camptothecin stability and efficacy *in vivo*, J. Control. Release 74 (2001) 243–247.
[136] R. Bhatt, P. de Vries, J. Tulinsky, G. Bellamy, B. Baker, J.W. Singer, P. Klein, Synthesis and *in vivo* antitumor activity of poly(L-glutamic acid) conjugates of 20(S)-camptothecin, J. Med. Chem. 46 (2002) 190–193.
[137] H. Uchino, Y. Matsumura, T. Negishi, F. Koizumi, T. Hayashi, T. Honda, N. Nishiyama, K. Kataoka, S. Naito, T. Kakizoe, Cisplatin-incorporating polymeric micelles (NC-6004) can reduce nephrotoxicity and neurotoxicity of cisplatin in rats, Br. J. Cancer 93 (2005) 678–687.
[138] F. Koizumi, M. Kitagawa, T. Negishi, T. Onda, S.-i. Matsumoto, T. Hamaguchi, Y. Matsumura, Novel SN-38-incorporating polymeric micelles, NK012, eradicate vascular endothelial growth factor-secreting bulky tumors, Cancer Res. 66 (2006) 10048–10056.
[139] T. Nakanishi, S. Fukushima, K. Okamoto, M. Suzuki, Y. Matsumura, M. Yokoyama, T. Okano, Y. Sakurai, K. Kataoka, Development of the polymer micelle carrier system for doxorubicin, J. Control. Release 74 (2001) 295–302.
[140] Y. Matsumura, T. Hamaguchi, T. Ura, K. Muro, Y. Yamada, Y. Shimada, K. Shirao, T. Okusaka, H. Ueno, M. Ikeda, N. Watanabe, Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin, Br. J. Cancer 91 (2004) 1775–1781.
[141] B. Twaites, C. de las Heras Alarcon, C. Alexander, Synthetic polymers as drugs and therapeutics, J. Mater.Chem. 15 (2005) 441–455.
[142] V. Delplace, P. Couvreur, J. Nicolas, Recent trends in the design of anticancer polymer prodrug nanocarriers, Polym. Chem. 5 (2014) 1529–1544.
[143] S. Zhang, J. Zou, M. Elsabahy, A. Karwa, A. Li, D.A. Moore, R.B. Dorshow, K.L. Wooley, Poly(ethylene oxide)-block-polyphosphester-based paclitaxel conjugates as a platform for ultra-high paclitaxel-loaded multifunctional nanoparticles, Chem. Sci. 4 (2013) 2122.
[144] Q. Yin, R. Tong, Y. Xu, K. Baek, L.W. Dobrucki, T.M. Fan, J. Cheng, Drug-initiated ring-opening polymerization of O-carboxyanhydrides for the preparation of anticancer drug–poly(O-carboxyanhydride) nanoconjugates, Biomacromolecules 14 (2013) 920–929.
[145] R. Tong, J. Cheng, Paclitaxel-initiated, controlled polymerization of lactide for the formulation of polymeric nanoparticulate delivery vehicles, Angew. Chem. Int. Ed. Engl. 47 (2008) 4830–4834.
[146] R. Tong, J. Cheng, Ring-opening polymerization-mediated controlled formulation of polylactide–drug nanoparticles, J. Am. Chem. Soc. 131 (2009) 4744–4754.
[147] R. Tong, J. Cheng, Drug-initiated, controlled ring-opening polymerization for the synthesis of polymer–drug conjugates, Macromolecules 45 (2012) 2225–2232.
[148] S. Manju, K. Sreenivasan, Synthesis and characterization of a cytotoxic cationic polyvinylpyrrolidone–curcumin conjugate, J. Pharm. Sci. 100 (2011) 504–511.
[149] S. Manju, K. Sreenivasan, Conjugation of curcumin onto hyaluronic acid enhances its aqueous solubility and stability, J. Colloid Interface Sci. 359 (2011) 318–325.
[150] S. Dey, K. Sreenivasan, Conjugation of curcumin onto alginate enhances aqueous solubility and stability of curcumin, Carbohydr. Polym. 99 (2014) 499–507.
[151] R. Yang, S. Zhang, D. Kong, X. Gao, Y. Zhao, Z. Wang, Biodegradable polymer–curcumin conjugate micelles enhance the loading and delivery of low-potency curcumin, Pharm. Res. 29 (2012) 3512–3525.
[152] W. Gao, J.M. Chan, O.C. Farokhzad, pH-responsive nanoparticles for drug delivery, Mol. Pharm. 7 (2010) 1913–1920.
[153] F. Meng, W.E. Hennink, Z. Zhong, Reduction-sensitive polymers and bioconjugates for biomedical applications, Biomaterials 30 (2009) 2180–2198.
[154] F.M. Veronese, O. Schiavon, G. Pasut, R. Mendichi, L. Andersson, A. Tsirk, J. Ford, G. Wu, S. Kneller, J. Davies, R. Duncan, PEG–doxorubicin conjugates: influence of polymer structure on drug release, *in vitro* cytotoxicity, biodistribution, and antitumor activity, Bioconjug. Chem. 16 (2005) 775–784.

[155] K. Matyjaszewski, Atom Transfer Radical Polymerization (ATRP): current status and future perspectives, Macromol. 45 (2012) 4015–4039.
[156] G. Moad, E. Rizzardo, S.H. Thang, RAFT polymerization and some of its applications, Chem. Asian. J. 8 (2013) 1634–1644.
[157] G.C. Vougioukalakis, R.H. Grubbs, Ruthenium-based heterocyclic carbene-coordinated olefin metathesis catalysts, Chem. Rev. 110 (2010) 1746–1787.
[158] N.E. Kamber, W. Jeong, R.M. Waymouth, R.C. Pratt, B.G. Lohmeijer, J.L. Hedrick, Organocatalytic ring-opening polymerization, Chem. Rev. 107 (2007) 5813–5840.
[159] V.P. Chauhan, R.K. Jain, Strategies for advancing cancer nanomedicine, Nat. Mater. 12 (2013) 958–962.