Available online at www.sciencedirect.com
ScienceDirect
Focus Article, *Mendeleev Commun.*, 2013, **23**, 179–186
Mendeleev Communications

Cross-linked polymeric micelles based on block ionomer complexes

Jong Oh Kim,[a] Thiruganesh Ramasamy,[a] Chul Soon Yong,[a] Natalia V. Nukolova,[b,c] Tatiana K. Bronich*[d] and Alexander V. Kabanov*[b,e]

[a] *College of Pharmacy, Yeungnam University, Gyongsan 712-749, South Korea*
[b] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*
[c] *Department of Fundamental and Applied Neurobiology, V. P. Serbsky National Research Center for Social and Forensic Psychiatry, 119991 Moscow, Russian Federation*
[d] *Department of Pharmaceutical Sciences and Center for Drug Delivery and Nanomedicine, College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198-5830, USA. Fax: +1 402 559 9365; e-mail: tbronich@unmc.edu*
[e] *Division of Molecular Pharmaceutics and Center for Nanotechnology in Drug Delivery, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7362, USA. E-mail: kabanov@unc.edu*

DOI: 10.1016/j.mencom.2013.07.001

Professor **Jong Oh Kim** received his Bachelor of Pharmacy and Master degrees in Pharmaceutics from the College of Pharmacy, Yeungnam University (South Korea) in 1997 and 2000, respectively. After he worked for Dong-A Pharmaceutical Research Lab. In 2000–2006, he joined PhD programme at the University of Nebraska Medical Center (Omaha, NE, USA). In 2010, he received PhD degree under the supervision of Prof. Tatiana K. Bronich at the University of Nebraska Medical Center. Since 2011, he is an assistant professor at the College of Pharmacy, Yeungnam University.

Thiruganesh Ramasamy received his Bachelor of Pharmacy from Pallavan Pharmacy College, Dr. M.G.R. Medical University in 2005 (Chennai, India). He received his Master degree from Vel's College of Pharmacy, Dr. M.G.R. Medical University in 2009. Thereafter, he worked for a brief period at the Pydah College of Pharmacy, Kakinada, India. Then, he was a research assistant in Utah-Inha DDS & Advanced Therapeutic Research Center under the supervision of Dr. Ziyad S. Haidar in 2010–2011. Since then, he is a graduate student under the guidance of Professor Jong Oh Kim and Professor Chul Soon Yong at the College of Pharmacy, Yeungnam University (South Korea).

Professor **Chul Soon Yong** graduated from the College of Pharmacy, Seoul National University in 1980. He received his PhD degree from the University of South Carolina in 1991. In 2008–2010, he was the dean of College of Pharmacy, Yeungnam University. Currently, he is the president of The Korean Society of Pharmaceutical Sciences and Technology and president-elect of The Asian Federation for Pharmaceutical Sciences (AFPS), and chair of Organizing Committee for AFPS Conference 2013. His research interests cover the development of drug delivery systems including drug targeting.

Dr. **Natalia V. Nukolova** graduated from the M. V. Lomonosov Moscow State University in 2004. In 2006–2010, she worked as a research assistant at the Center for Drug Delivery and Nanomedicine at the University of Nebraska Medical Center. In 2010, she received her PhD in polymer chemistry from the M. V. Lomonosov Moscow State University under the supervision of Dr. Alexander V. Kabanov. Since 2011, she is a senior staff scientist at the V. P. Serbsky National Research Center for Social and Forensic Psychiatry in Moscow, Russia. Her major interest is the development of new approaches to the diagnosis and treatment of cancer.

Professor **Tatiana K. Bronich** received her PhD degree in polymer chemistry from the M. V. Lomonosov Moscow State University in 1986. She continued research in the area of synthetic polyelectrolytes and biological macromolecules at the Institute of Organoelement Compounds of the Russian Academy of Sciences, the Department of Chemistry at the Moscow State University and then joined the laboratory of Dr. Alexander V. Kabanov in Omaha, Nebraska. She is now the Parke Davis Professor at the College of Pharmacy and Co-Director of the Center for Drug Delivery and Nanomedicine at the University of Nebraska Medical Center. Her recent work has expanded to include the application of amphiphilic block copolymers and block ionomer complexes in drug delivery to treat cancer and infectious diseases.

Professor **Alexander V. Kabanov** graduated and received his PhD and Dr. Sci. degrees from the M. V. Lomonosov Moscow State University. He is a Mescal S. Ferguson Distinguished Professor, Director of the Center for Nanotechnology in Drug Delivery, and Co-Director of the Carolina Institute for Nanomedicine at the University of North Carolina at Chapel Hill, USA since 2012. Prior to this appointment, he served for nearly 18 years at the University of Nebraska Medical Center where he founded the Center for Drug Delivery and Nanomedicine and became its first director in 2004. He is also a professor at the M. V. Lomonosov Moscow State University where he founded the Laboratory of Chemical Design of Bionanomaterials with the 'Megagrant' support from the Russian Government in 2010. Dr. Kabanov has conducted pioneering research on polymeric micelles, DNA/polycation complexes, block ionomer complexes and nanogels for delivery of small drugs, nucleic acids and proteins that considerably influenced current ideas and approaches in drug delivery and nanomedicine.

Polymeric micelles formed as a result of the self-assembly of block copolymers constitute an important class of supramolecular structures that attracted attention as drug carriers in drug delivery and nanomedicine. Chemical stabilization of the polymeric micelles by introducing cross-links between block copolymer chains can increase stability and prevent premature disintegration of these micelles in the body. The cross-linking strategy has been also applied to a special class of polymeric micelles formed by block copolymers with ionic and nonionic water-soluble segments (block ionomers) that are electrostatically coupled with oppositely charged species. Such polyion complex micelles (also termed block ionomer complexes) have high colloidal stability due to steric repulsion of their corona and often assume a spherical core–shell morphology. These species are currently actively researched for the delivery of various low molecular weight drugs and protein or nucleic acid-based therapeutics. This paper highlights recent advances in the development of cross-linked polymeric micelles, the physicochemical aspects of the formation and behavior of various block ionomer complexes, the strategies for the cross-linking of core and shell parts of the micelles, and the advantages and major challenges in their biomedical applications.

The development of safe and efficient carriers for the delivery of therapeutically active molecules is necessary for the improvement of current therapies and the implementation of novel therapeutic modalities. Research in this area has risen enormously over the past decade with numerous drug carriers proposed. However, their therapeutic approval continues to be hindered by various shortfalls.[1,2] In this context, polymer-based drug carriers have appealing chemical and biological characteristics, such as good biocompatibility and low immunogenicity, which make them well-suited for prospective clinical applications.[3] Advances in synthetic polymer chemistry enable versatile ways to functionalize and customize the structure of therapeutic polymers to adapt them to different therapeutic regimens. In particular, the ability to finely tune the polymer hydrophilicity/lipophilicity, make polymers biodegradable, and add cell-targeting moieties to polymer-based carriers is the major advantage of such systems.[4]

Among various innovative polymer-based drug delivery systems, polymeric micelles have attracted tremendous attention in recent years. Such systems are formed by amphiphilic block copolymers with hydrophobic and hydrophilic blocks, which self-assemble in selective solvents into polymeric micelles (10 to 100 nm) having fairly narrow size distributions. These micelles have unique core–shell architectures with hydrophobic polymer blocks segregating into a micelle core surrounded by a shell of hydrophilic blocks. The core–shell architecture of the polymeric micelles is essential for their utility as novel functional materials for pharmaceutical applications.[5–9] The core of the micelles is a cargo space that accommodates various therapeutic or diagnostic agents, and the shell stabilizes the micelles in aqueous dispersion.

Poly(ethylene oxide) (PEO) is frequently used as a hydrophilic block of micelle-forming copolymers since this polymer is highly hydrated, soluble, low-toxic and low-immunogenic; it can serve as an efficient steric stabilizer for nanoparticles in biological media.[10,11] In particular, PEO chains grafted to the surface of nanoparticles prevent the interactions of these nanoparticles with serum proteins and cells, inhibit particle opsonization and render the particles less 'recognizable' by the reticuloendothelial system (RES) in the liver and spleen. Since the molecular weight of polymeric micelles is far above the renal threshold (~40 kDa for polymers), they are believed to evade renal excretion and nonspecific capture by the RES, and demonstrate prolonged circulation times in blood. Recent developments and applications of block copolymer micelles have been extensively reviewed.[5–9]

The field of polymeric micelles was significantly advanced by employing charge driven self-assembly of block copolymers containing water-soluble ionic and nonionic blocks (block ionomers or double hydrophilic block copolymers). The idea of using the block ionomers to design novel nanocomposite materials was first proposed by a team led by A. Kabanov, V. Kabanov and A. Eisenberg in Russia, the United States and Canada[12,13] and independently by K. Kataoka and colleagues in Japan.[14] This scientific field was driven by a necessity for the development of advanced delivery systems with a core–shell architecture that could incorporate biopolymers such as polynucleotides, proteins and other ionic components. In the absence of oppositely charged molecules, block ionomers are fully soluble in aqueous solution. However, their micellization can be induced by adding oppositely charged molecules such as synthetic linear polyelectrolytes,[13] block ionomers,[15] surfactants/lipids,[16,17] proteins,[18,19] DNA[20] or metal ions.[21,22] These molecules form electrostatic complexes with the charged blocks of block ionomers, and, due to charge neutralization, prompt spontaneous segregation of the resulting complexes into micelle-like structures with electrostatically neutralized polyion cores and hydrophilic polymer shells. Such structures are known as block ionomer complexes (BICs) or polyion complex (PIC) micelles[23–25] (Figure 1). It was demonstrated that BICs vastly differ from the regular inter-polyelectrolyte complexes (IPECs) formed by homopolymers or random copolymers of opposite charge. In particular, even though the charges of polyion blocks are completely neutralized, BICs form stable aqueous dispersions due to the steric stabilization effect of hydrophilic nonionic blocks that prevent aggregation and macroscopic phase separation. Unique features of such BICs allow for the delivery of biomacromolecules, as well as ionic and nonionic drugs. A variety of compounds of pharmaceutical and biomedical relevance have been incorporated through a combination of electrostatic, hydrophobic and hydrogen bonding interactions in such complexes.[26,27]

Although BIC-based polymeric micelles are stable in dispersion, their stability can be affected by variations in pH, temperature, salt concentration and the chemical nature of low molecular weight counterions. After administration in the body, the dissociation of BICs to individual polymer chains is inevitable due to dilution in blood, gastric, or other body fluids, and that hampers their practical applications. Charged molecules present in the blood such as serum proteins can often displace the ionic components in the BIC. For many applications, it is advantageous to produce a covalently stabilized nanostructure, which is robust and maintains its integrity under various conditions. The most effective way to achieve this is to covalently interconnect the polymer chains of the block ionomers by cross-linking either the shell or core parts of BIC micelles. Thus, the objective of this article is to present an overview of cross-linked polymeric micelles based on BIC.

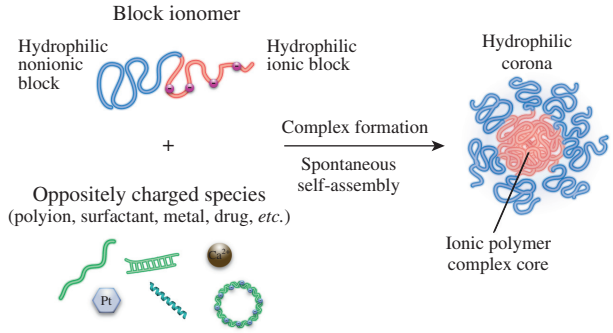

Figure 1 Schematic diagram of the formation of block ionomer complexes.

Cross-linked BIC micelles

The colloidal stability of BIC is highly dependent on the environmental parameters such as pH and ionic strength. From a practical point of view, a low structural stability of BIC micelles in biological surroundings could be problematic for their biomedical applications. Indeed, polymeric micelles have a limited lifetime and disintegrate after dilution in body fluids, which results in premature drug release.[28] Thus, there is a significant interest in the stabilization of BIC micelles, which can be achieved by introducing covalent cross-links between the polymer chains. Covalent stabilization can reinforce the multimolecular assembly of the micelles and prevent their structural deterioration upon dilution and environmental challenges such as acidic pH, physiological ionic strength and shear forces.

There are numerous reports on the stabilization of polymeric micelles by cross-linking either within the core domain or throughout the corona layer. One of the first attempts to stabilize micellar structures was made by Prochaska and Baloch and involved the cross-linking of the polybutadiene core of polystyrene-*b*-polybutadiene micelles by photochemical irradiation.[29] Subsequently, the UV irradiation of photoreactive poly(cinnamoylethyl methacrylate) blocks in the core of polystyrene-poly(cinnamoylethyl methacrylate) micelles locked their structures and did not lead to significant changes in the aggregation number of the micelles.[30] Since then, many alternative strategies to introduce cross-links into the core domain[31,32] or corona layer[33–36] of the micellar constructs have been proposed (Figure 2). The resulting cross-linked polymeric micelles maintained small size and core–shell morphology while their dissociation was suppressed. For example, Iijima *et al.*[32] have shown that core cross-linked micelles of PEO-*b*-poly(D,L-lactide) possess high stability against dilution, temperature change, and even dissolution of surfactants, which proved that core cross-linking is an effective way to avoid micellar disintegration upon dilution. Wooley and co-workers[33–36] cross-linked the micellar corona and obtained the so-called shell cross-linked knedel-like micelles (SCKs) – robust nanostructures with a permeable cross-linked shell. Similarly, Rijcken *et al.*[37] reported a core cross-linked (CCL) micelles based on $mPEG_{5000}$ and *N*-(2-hydroxyethyl)methacrylamide oligolactates [mPEG-*b*-p(HEMAm-Lac_n)]. They reported an enhanced stability in physiological pH, enhanced tumor uptake and reduced liver uptake of CCL micelles in comparison with those of non-cross-linked (NCL) micelles. This could be due to dissociation of NCL micelles after intravenous administration and/or were opsonized and captured by macrophages while the dense PEG shell of CCL micelles made them less prone towards opsonization; this will favor their biodistribution profile.[37]

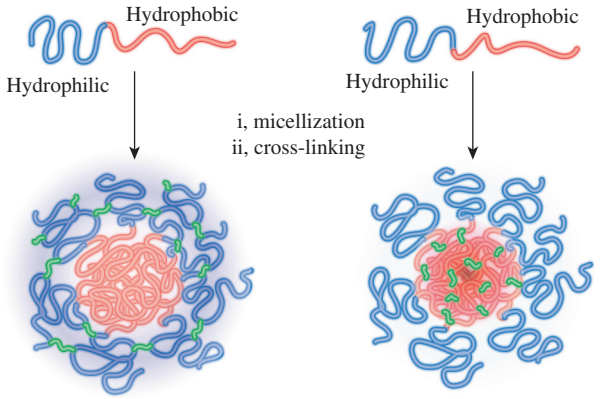

Figure 2 Schematic diagram of different functional amphiphilic block copolymers utilized in the formation of shell cross-linked or core cross-linked polymeric micelles.

Using similar strategies, one can also enhance the structural stability of BIC micelles against changes under external conditions. Bronich and co-workers developed a unique procedure, in which BIC micelles were initially prepared by the self-assembly of ionic blocks of water-soluble block ionomers [poly(ethylene oxide)-*b*-poly(methacrylic acid) (PEO-*b*-PMA)] with an oppositely charged condensing agent (*e.g.*, Ca^{2+} or Ba^{2+}). This was followed by the chemical cross-linking of ionic blocks in the core and the removal of a condensing agent.[19–21,28] The resulting cross-linked PEO-*b*-PMA micelles represented pH-dependent hydrogel-like soft nanospheres with cores comprised of a network of the cross-linked polyanions surrounded by a flexible hydrophilic shell composed of PEO blocks (Figure 3). The micelles are very stable, and they can be freeze-dried and reconstituted in aqueous dispersion. A similar template-assisted technique was used to prepare a core-corona type of BIC micelles through the condensation and cross-linking of comb-graft poly(ethylene oxide)-*b*-poly(polypropylene oxide)-*b*-poly(ethylene oxide)-*g*-poly(acrylic acid) (Pluronic-PAA) copolymers.[28] The swelling of the cross-linked micelles strongly depends on the degree of cross-linking and structure of cross-linker. Increasing the degree of cross-linking and hydrophobicity of cross-linkers resulted in a decreased swelling ratio of the cross-linked micelles.[38,39] The introduction of reversible cross-links into swollen hydrophilic micelles allowed developing *in situ* degradable micelles that have an advantage of potential elimination from the body by renal excretion.[39] Importantly, drug-loaded polymeric micelles with degradable cross-links exhibited more potent cytotoxicity against cancer cells compared to nondegradable micelles.[38–40] Unexpectedly, these micelles displayed selective entry into cells *via* caveolae-mediated endocytosis.[40] Their entry was inhibited in epithelial

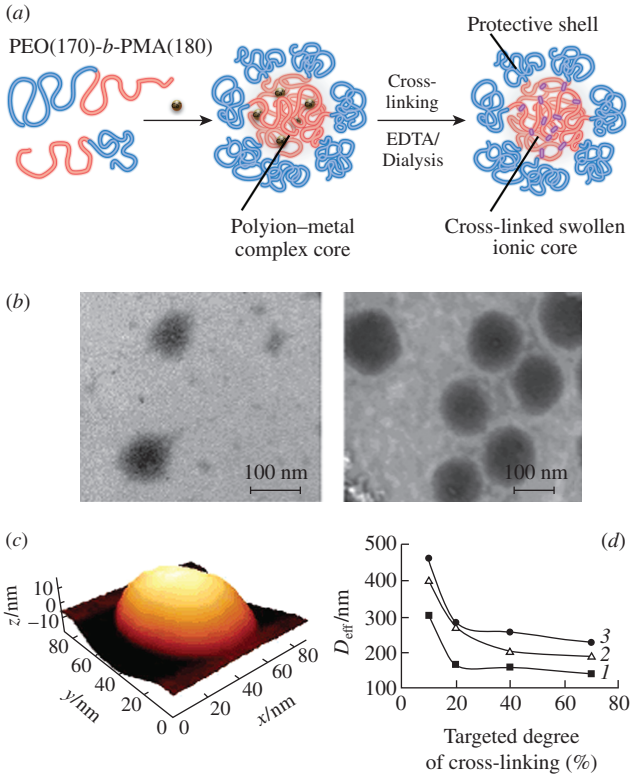

Figure 3 (*a*) Synthesis of the cross-linked polymeric micelles. (*b*) Transmission electron microscopy (TEM) images of PEO-*b*-PMA/Ca^{2+} (left) and cross-linked polymeric (right) micelles. (*c*) Tapping-mode atomic force microscopy (AFM) image of a cross-linked polymer micelle in air. (*d*) The effective diameter (D_{eff}) of cross-linked PEO-*b*-PMA micelles at various pH as a function of targeted degree of cross-linking. (*1*) pH 5.5, (*2*) pH 7.8, and (*3*) pH 9.6. Reproduced with permission from ref. 21, © 2005 American Chemical Society, and ref. 22, © 2006 Elsevier.

cells forming tight junctions, but permitted in cancer cells that do not form tight junctions. Thus, cancer cells contrary to normal cells internalized cross-linked polymeric micelles resulting in their transport to acidic compartments (lysosomes). Under conditions of low pH, polymeric micelles released drugs entrapped in them and killed cancer cells. Such polymeric micelles did not kill normal cells since they did not penetrate into them but got entrapped outside in the areas of tight junctions. Thus, the unique ability of such cross-linked polymeric micelles to interact with caveolae makes it possible to selectively kill cancer cells and sparing normal cells. This can lead to the development of drug delivery systems with reduced side effects and higher efficacy in cancer chemotherapy.[40]

Kataoka's group developed reversibly cross-linked BIC micelles containing disulfide bonds [Figure 4(*a*)]. Such cross-links are stable in extracellular fluid, however, cleaved in the reducing environment of cytoplasm owing to differences in the reductive potential between extracellular and intracellular compartments. Polymeric carriers based on this approach have been successfully utilized in the delivery of anticancer drugs,[41,42] imaging agents,[43] plasmid DNA and antisense oligonucleotides.[44–49] For instance, BIC formed by thiolated PEO-*b*-poly(L-lysine) (PEO-*b*-PLL) copolymers and oppositely charged molecules such as polyanions, plasmid DNA, antisense oligo-DNA or siRNA were prepared.[45–49] Assembled, spherical, nanosized BIC micelles were further cross-linked by the oxidation of thiol residues introduced into the PLL blocks, resulting in the formation of disulfide bonds in the cores of the micelles. The dissociation of PEO-*b*-PLL based BIC was inhibited by such environmentally sensitive cross-linking while complexes were destabilized after reductive cleavage at the target site.[50]

McCormick and co-workers also reported reversible shell cross-linked micelles, which were synthesized by the cross-linking of *N*-acryloxysuccinimide units incorporated within a thermoresponsive triblock copolymer (PEO-*b*-[poly(*N*,*N*-dimethylacrylamide)-stat-(*N*-acryloxysuccinimide)]-*b*-(*N*-isopropylacrylamide) [PEO-*b*-(DMA-*s*-NAS)-*b*-NIPAM] with cystamine, a bifunctional cross-linker, which contains a disulfide bond [Figure 4(*b*)].[51] These micelles were reversibly cleaved using either dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and re-cross-linked repeatedly using cystamine. Bronich *et al.* demonstrated that the formation of disulfide bonds between surfactant molecules in BIC formed by PEO-*b*-PMA and reactive single-tail surfactant, isothiuronium methyl hexadecyl dimethyl ammonium bromide (C_{16}SU), resulted in the formation of vesicles, which were stable in a broad range of environmental conditions (pH, temperature and salt concentration).[52] Furthermore, they have successfully demonstrated time-dependent degradation under the conditions mimicking the intracellular reducing environment. Disulfide bonds were introduced into the ionic cores of PEO-*b*-PMA-based cross-linked micelle using cystamine as a biodegradable cross-linker. Due to the ionic character of the cores, a very high level of drug loading into the cross-linked polymeric micelles was achieved. These cross-linked polymeric micelles were shown to accelerate drug release in the presence of reducing agents.[39]

'Click' chemistry was recently utilized for the covalent stabilization of BIC micelles. Self-assembled BIC micelles were prepared by reacting negatively charged poly(methacrylic acid-co-3-azidopropyl methacrylate)-*g*-poly(*N*-isopropylacrylamide [P(MAA-co-AzPMA)-*g*-PNIPAM] and positively charged poly-[2-(dimethylamino)ethylmethacrylate-co-AzPMA-*g*-PNIPAM] [P(QDMA-co-AzPMA)-*g*-PNIPAM] copolymers containing azide groups.[53,54] Subsequently the polyion was cross-linked *via* 'click' reactions with propargyl ether, a bifunctional cross-linker (Figure 5). Compared to non-cross-linked micelles, the 'click' core cross-linked BIC micelles improved stability upon salt addition and pH changes. Moreover, thermo-responsive PNIPAM coronas induced temperature-dependent aggregation of the BIC.[53]

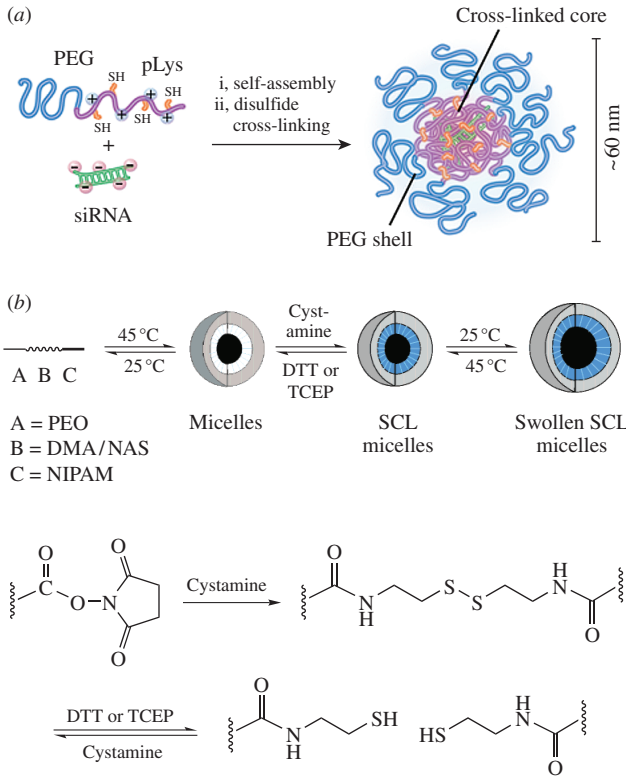

Figure 4 (*a*) Environment sensitive stabilization of BIC micelles through the formation of a disulfide bond in the core. (*b*) Schematic illustration of the formation of reversible shell cross-linked (SCL) micelles from PEO-*b*-(DMA-*s*-NAS)-*b*-NIPAM triblock copolymers. Reproduced with permission from ref. 51. © 2006 American Chemical Society.

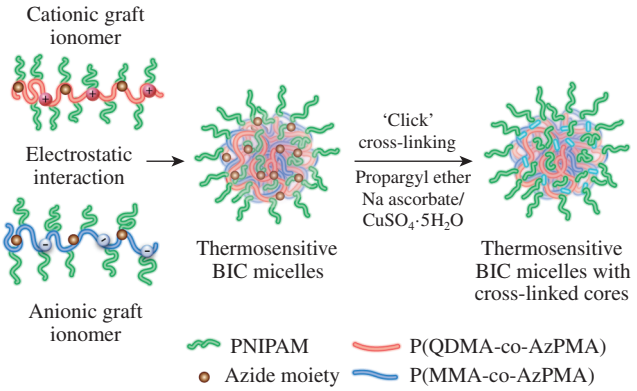

Figure 5 Schematic diagram of the formation of thermosensitive BIC micelles and their core cross-linking *via* click chemistry.

Biomedical applications of cross-linked BIC micelles

Gene and siRNA delivery. A large number of non-viral gene delivery systems based on the polyelectrolyte complexes of DNA (polyplexes) have been designed and tested for their safety and efficacy *in vitro* and *in vivo*. However, clinical success of such systems is hampered by their inefficient cellular delivery, which is largely influenced by carrier properties.[55,56] Presently, cationic block and graft copolymers have attracted increasing attention as carriers for polynucleotides. In this case, the neutralization of a positive charge on the polycation block by negatively charged DNA or siRNA leads to the formation of BIC micelles. Advanced and tunable characteristics of the cationic copolymers such as the chemical structure and length of segments, rigidity, hydrophilicity, charge density and biodegradability allow one to modulate gene-

delivery properties such as DNA binding, colloidal stability of BIC, toxicity, endosomal escape, vector unpacking and transfection efficiency.

The cross-linking strategy has been used to further increase the stability of BIC micelles against dissociation and to maintain their ability to bind DNA. For instance, the reversible cross-linking of polyplexes is especially attractive for targeted degradation of polyplexes in the intracellular environment because the concentration of glutathione (a natural disulfide reducing agent) is about 50–1000 times higher inside cells compared to the extracellular environment.[54] Cross-linked polyplexes prepared with thiol-modified PEO-*b*-PLL copolymers have shown both increased transfection using pDNA and increased gene knockdown using siRNA.[45] Thiol-modified polycations have shown the ability to increase the efficacy of nucleic acid delivery systems due to stabilization of the polyplex by disulfide formation. Similarly, disulfide cross-linked PEO-*g*-polyethyneimine (PEO-*g*-PEI) polyplexes with the amine reactive cross-linker dithiobis(succinimidyl propionate) significantly increased the mechanical stability and improved blood level profiles of DNA by preventing premature release of complexed DNA.[57] Kataoka's group incorporated pDNA in BIC with thiolated c(RGDfK)-PEO-*b*-PLys block copolymer. The resulting BIC micelles carried cyclic RGD peptide ligands at the surface and exhibited remarkably high transfection efficiency against HeLa cells expressing integrins on the surface.[50] In a subsequent study, Vachutinsky *et al*. cross-linked BIC micelles with RGD peptides through ion complexation of thiolated c(RGDfK)-poly(ethylene glycol)-*b*-poly(L-lysine) [c(RGDfK)-PEO-P(Lys-SH)] and pDNA. They reported an increased tumor accumulation, increased interaction with endothelial cells, enhanced intracellular uptake and enhanced anti-angiogenic activity of the pDNA resulting from its complexation with thiolated c(RGDfK)-PEO-P(Lys-SH).[58] Using cross-linked thiolated PEO–polyphosphoramidates polyplexes, Jiang *et al*. showed prolonged expression of luciferase pDNA in HEK293 cells, which was attributed to the slow unpacking of the DNA in the presence of glutathione.[59]

Protein-containing BIC. BIC can also be obtained if a polyelectrolyte is a protein or polypeptide. Indeed, when pH of the solution exceeds the isoelectric point, a protein becomes charged and can form protein-polyelectrolyte complexes with oppositely charged block ionomers. Kataoka *et al*. have shown that the incorporation of proteins into BIC can be beneficial for effective protein delivery. Egg white lysozyme (isoelectric point pI = 11) was first examined for incorporation into the core of the BIC micelles using an anionic PEO-poly(α,β-aspartic acid)(PEO-*b*-PAsp).[60] The same group has formulated positively charged lysozymes with negatively charged PEO-*b*-PAsp block copolymer. Importantly, the apparent enzymatic activity of lysozyme in BIC was not only retained but even enhanced compared to that of the native enzyme.[61,62] It was hypothesized that the elevated enzymatic activity is a result of substrate concentration in the polyion complex species and/or alterations in binding mode between lysozyme and substrate.[60,63] This group prepared BIC micelles from PEO-*b*-PAsp and trypsin. These complexes were cross-linked with glutaraldehyde through the Schiff base formation.[64] The stability of the resulting BIC was significantly improved by introducing cross-links in the core.

Recently, A. Kabanov's group prepared nanosized BIC of antioxidant enzymes by self-assembly of Cu/Zn superoxide dismutase (SOD1) or catalase with oppositely charged cross-linked block ionomers ('nanozymes') followed by covalent cross-linking to further stabilize nanoparticles (Figure 6). They demonstrated an increased stability of cross-linked nanozymes in both blood and brain and increased accumulation in central nervous system (CNS) in comparison with non-cross-linked complexes and

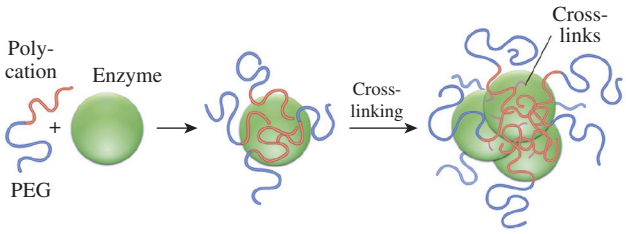

Figure 6 Cross-linked nanozymes containing SOD1. Reproduced with permission from ref. 65. © 2012 Elsevier.

native SOD1.[65] The therapeutic efficacy of such SOD1 BICs has been shown in the middle cerebral artery occlusion model (MCAO) of stroke.[66] Similarly, Batrakova *et al*. developed a bone-marrow-derived macrophage (BMM) system to deliver catalase to Parkinson's disease-affected brain regions in a mouse model. This has been accomplished by packaging catalase into BIC with a cationic block copolymer, PEO-*g*-PEI. The self-assembled catalase/PEO-*g*-PEI BIC were stable at physiological pH and ionic strength, and retained antioxidant activities.[18] Importantly, the incorporation of catalase into BIC precluded the enzyme degradation inside the host cells and allowed for delivery of active enzyme to the brain. SOD1 packed in BIC with PEO-*g*-PEI copolymer can enter neurons, resulting in increased delivery of functional SOD1 to neurons and inhibited the angiotensin II intra-neutronal signaling.[67] In addition, butyrylcholinesterase (BChE) protein was delivered to CNS by packing it in BIC of cationic PEO-*g*-PLL copolymer.[68] Heffernan and Murthy have designed PEO-*b*-PLys block copolymer for the delivery of ovalbumin and immunostimulatory CpG-DNA through disulfide reduction.[69] To date, several other proteins such as trypsin,[70] myoglobin,[19] bovine serum albumin (BSA),[71] and cytochrome C[72] have been incorporated into BIC micelles to increase their stability and functional activity. Stability of enzyme-incorporated BIC was further improved by introducing hydrophobic groups such as phenyl to block copolymer[73] or by the cross-linking of the cores of micelles.[70]

Drug-containing cross-linked BIC. Cross-linked polymeric micelles with a biodegradable or nonbiodegradable main chain can be employed for controlled drug delivery when the micelles are able to dissociate through the degradation of cross-links. Lee *et al*. encapsulated camptothecin (CPT) in the cores of cross-linked poly(D,L-lactic acid)/poly(ethylene glycol)/poly(D,L-lactic acid) (PLA-PEO-PLA) micelles and showed sustained release of the drug.[74] Similarly, Stenzel's group investigated poly(propargyl methacrylate)-based micelles that were core cross-linked with a reducible disulfide linker and complexed with the anticancer drug hexacarbonyl dicobalt.[75] The drug-loaded cross-linked micelles exhibited a significant reduction in drug-related cytotoxicity compared to non-cross-linked micelles. Li *et al*.[76] reported that drug release from thermoresponsive shell cross-linked poly(ethylene oxide)-*b*-[(*N*,*N*-dimethylacrylamide)-stat-(*N*-acryloxysuccinimide)]-*b*-(*N*-isopropylacrylamide) [PEO-*b*-(DMA-*s*-NAS)-*b*-NIPAM] micelles prepared by using reversible cystamine cross-linkers can be tuned by altering the chemical nature and concentration of a reducing agent (*e.g*., 35 and 50% releases were observed in the absence and presence of DTT, respectively).[76] Similarly, doxorubicin-loaded poly(ethylene oxide)-*b*-poly(caprolactone) (PEO-*b*-PCL) block copolymer micelles, which were cross-linked by lipoic acid esters, exhibited varied drug release upon the exposure to the reducing agent (<15% release in 10 h and 75% in 9 h upon expose to DTT).[77]

Anticancer drugs such as *cis*-dichlorodiammineplatinum(II) (CDDP) or doxorubicin (Dox) were successfully loaded into BIC micelles by metal complexation or electrostatic interactions. Block

ionomer-metal complexation of CDDP with PEO-poly(amino acid) block copolymers such as PEO-*b*-PAsp or polyethylene oxide-*b*-polyglutamate (PEO-*b*-PGlu) led to the spontaneous formation of stable BIC micelles.[78,79] Sustained release of platinum compounds was observed *via* an exchange reaction between chloride ions and the carboxylic groups of block ionomers in platinum complexes. In particular, PEO-*b*-PGlu/CDDP micelles showed prolonged blood circulation and effective accumulation in solid tumors by the enhanced permeation and retention (EPR) effect, resulting in complete tumor regression with a minimal body weight loss.[79,80] Similar approach was utilized for the incorporation of other platinum-containing drug, dichloro(1,2-diaminocyclohexane)platinum(II) (DACHPt), the oxaliplatin analog, into BIC through polymer-metal complex formation between DACHPt and PEO-*b*-PGlu.[81] The DACHPt-loaded micelles exhibited considerably higher stability compared to the CDDP-loaded micelles in spite of their comparable release rate. Such a behavior was attributed to the hydrophobic nature of DACHPt complexes in the micellar core.

The stability of block ionomer/drug complexes and loading capacity for drugs were considerably improved by cross-linking the BIC micelles using bifunctional cross-linkers.[39,64,82] It was shown that Dox release from BIC micelles with cross-linked ionic cores can be modulated in a pH or salt-dependent fashion. Introduction of disulfide bonds in the cross-linked ionic core of BIC micelles potentiated *in vitro* cytotoxicity of drugs.[33] CDDP was also successfully immobilized into ionic cores of hydrophilic cross-linked micelles.[17] Oberoi *et al*. have shown that the loading capacities and release profile of CDDP can be tailored by altering the degree of cross-linking.[83] Further, the drug was released in a pH-dependent manner without loss of its biological activity.[83] In order to increase the intracellular concentration of anti-cancer drug and cell uptake, the PEO-*b*-PMA block copolymer was decorated with folate residues and the core cross-linked micelles were loaded with different drugs (CDDP and Dox) (Figure 7). The folate decorated cross-linked micelles effectively delivered CDDP to xenograft tumor expressing folate receptor and displayed superior antitumor activity *in vivo* along with decreased renal toxicity.[84] The promise of cross-linked micelles for drug delivery is further demonstrated by Talleli *et al*. who encapsulated Dox into core-cross-linked biodegradable polymeric micelles composed PEO-*b*-poly[*N*-(2-hydroxypropyl)methacrylamide lactate] [mPEO-*b*-p(HPMAm-Lac$_n$)] diblock copolymers. These authors demonstrated prolonged circulation of the micelles in the blood stream upon intravenous administration and enhanced tumor accumulation through the EPR effect.[85]

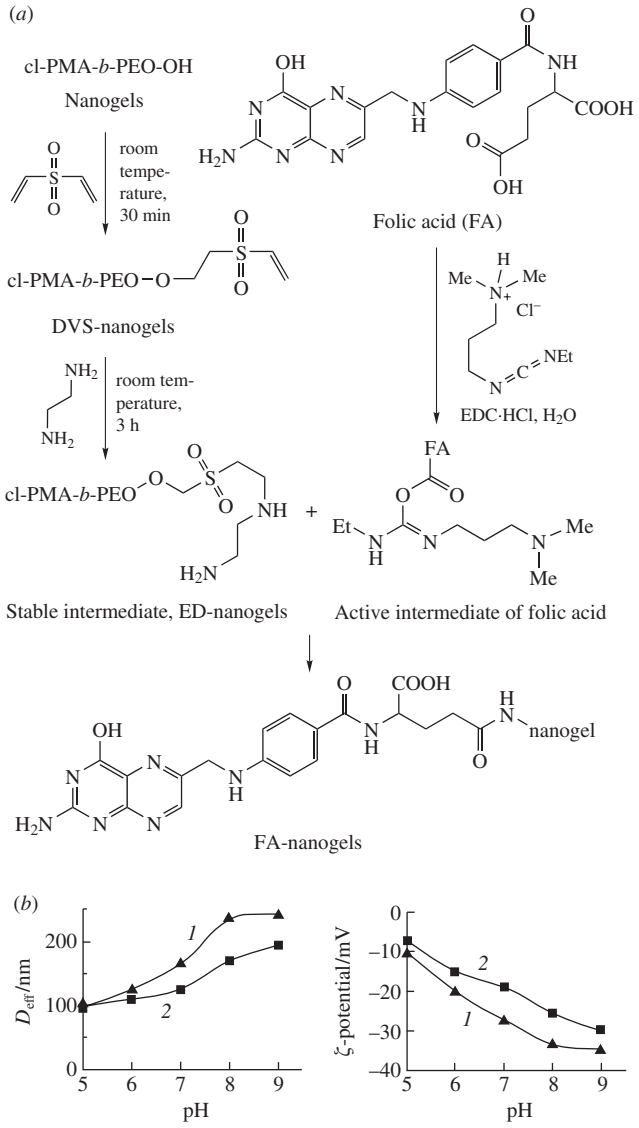

Figure 7 (*a*) Synthetic scheme for the preparation of FA nanogels. (*b*) Swelling behavior of nanogels and FA nanogels. The effective diameter (D_{eff}) and ζ-potential of (*1*) nanogel and (*2*) FA nanogel as a function of pH. Reproduced with permission from ref. 84, © 2011 Elsevier.

Advantages and challenges

BIC micelles are supramolecular nanoassemblies, which are formed through the self-assembly of block copolymers. Owing to their unique core–shell architecture, they are of high pharmaceutical significance. The cross-linked core of the micelles creates a large space for the accommodation of small molecule drugs, proteins or DNA. The hydrophilic shell, on the other hand, protects the drug-loaded core from the biological milieu. Further, the hydrophilic shell minimizes protein adsorption on micelles and their cellular adhesion, and thus evades nonspecific capture by the reticuloendothelial system. Many studies have demonstrated their structural integrity and prolonged circulation ability due to core–shell cross-linking. Furthermore, the control and versatility of polymer chemistry allows designing a broad range of drug formulations. The integrating of targeting ligand moieties onto the surface of cross-linked micelles can further facilitate their selective accumulation in the target tissue or cells. This can further pave ways for their potential use as sustained release nanocarriers for the delivery of therapeutic agents.

Despite having numerous advantages, the cross-linked polymeric micelles are yet to move to the next stage, and still its transition from bench to bedside remains a tough challenge for the scientists. The major hurdles include but not limited to their clearance from the body, cytotoxicity of cross-linking agents, preserving the nature of drug while cross-linking the BIC micelles, scale up feasibility *etc*. Furthermore, the instant heterogeneity of polymer micelles and difficulties with the manufacturing of batch-to-batch identical materials might hamper their progress to the clinical evaluation. In our view, these problems and challenges will be resolved through a focused clinical translation work that is underway in numerous laboratories.

Conclusions

As it stands now, polymer nanotechnology is rapidly expanding. Block copolymer micelles hold great promise for drug delivery applications, primarily due to their distinct core–shell morphology and potential for functionalization. However, the main problem is their low structural stability in biological surroundings, which causes the incorporated drugs to be quickly released upon intravenous administration. This is especially undesirable for cancer therapy due to the high toxicity and severe side effects

caused by chemotherapeutics. Intermolecular cross-linking is a promising approach to improve the stability of polymeric micelles. These cross-linked polymeric micelles have high colloidal stability *in vivo* due to the steric repulsion of their corona and often assume a spherical core–shell morphology. They also allowed the encapsulation of various charged therapeutic molecules, including large entities such as proteins and nucleic acids, into the micellar core. These nanocarriers have a high loading capacity with the ionic block providing for pH and salt sensitivity to control the triggered release of a therapeutic agent. Taken together, the cross-linked polymeric micelles are attractive carriers for the delivery of therapeutic agents.

This work was supported by the US National Science Foundation [grant nos. DMR0513699 and DMR0071682 (AVK)], the US National Institutes of Health [grant nos. 1R01CA116590 (TKB) and 1P20RR021937 (AVK/TB)], the US Department of Defense [grant no. USAMRMC 06108004 (AVK)] and the Government of the Russian Federation [grant no. 11.G34.31.0004 (AVK)]. We are grateful to Dr. D.Y. Filonova (Alakhova) for her assistance in the preparation of illustration for this paper.

References

1 D. W. Pack, A. S. Hoffman, S. Pun and P. S. Stayton, *Nat. Rev. Drug Discovery*, 2005, **4**, 581.
2 T. Merdan, J. Kopecek and T. Kissel, *Adv. Drug Delivery Rev.*, 2002, **54**, 715.
3 L. S. Nair and C. T. Laurencin, *Adv. Biochem. Engin./Biotechnol.*, 2006, **102**, 47.
4 D. Mishra, H. C Kang and Y. H. Bae, *Biomaterials*, 2011, **32**, 3845.
5 G. Riess, *Prog. Polym. Sci.*, 2003, **28**, 1107.
6 J.-F. Cohy, *Adv. Polym. Sci*., 2005, **190**, 65.
7 A. Rösler, G. W. M. Vandermeulen and H. A. Klok, *Adv. Drug Delivery Rev.*, 2001, **53**, 95.
8 S. R. Croy and G. S. Kwon, *Curr. Pharm. Des.*, 2006, **12**, 4669.
9 C. Allen, D. Maysinger and A. Eisenberg, *Colloids Surf. B: Biointerfaces*, 1999, **16**, 3.
10 G. S. Kwon and K. Kataoka, *Adv. Drug Delivery Rev*., 1995, **16**, 295.
11 M. Tobio, R. Gref, A. Sanchez, R. Langer and M. J. Alonso, *Pharm. Res*., 1998, **15**, 270.
12 A. V. Kabanov, S. V. Vinogradov, Y. G. Suzdaltseva and V. Y. Alakhov, *Bioconjugate Chem*., 1995, **6**, 639.
13 A. V. Kabanov, T. K. Bronich, V. A. Kabanov, K. Yu and A. Eisenberg, *Macromolecules*, 1996, **29**, 6797.
14 A. Harada and K. Kataoka, *Macromolecules*, 1995, **28**, 5294.
15 P. S. Chelushkin, E. A. Lysenko, T. K. Bronich, A. Eisenberg, V. A. Kabanov and A. V. Kabanov, *J. Phys. Chem. B*, 2008, **112**, 7732.
16 T. K. Bronich, A. V. Kabanov, V. A. Kabanov, K. Yu and A. Eisenberg, *Macromolecules*, 1997, **30**, 3519.
17 A. V. Kabanov, T. K. Bronich, V. A. Kabanov, K. Yu and A. Eisenberg, *J. Am. Chem. Soc.*, 1998, **120**, 9941.
18 E. V. Batrakova, S. Li, A. D. Reynolds, R. L. Mosley, T. K. Bronich, A. V. Kabanov and H. E. Gendelman, *Bioconjugate Chem.*, 2007, **18**, 1498.
19 A. Kishimura, A. Koide, K. Osada, Y. Yamasaki and K. Kataoka, *Angew. Chem. Int. Ed*., 2007, **46**, 6085.
20 M. Harada-Shiba, K. Yamauchi, A. Harada, I. Takamisawa, K. Shimokado and K. Kataoka, *Gene Ther.*, 2002, **9**, 407.
21 T. K. Bronich, P. A. Keifer, L. S. Shlyakhtenko and A. V. Kabanov, *J. Am. Chem. Soc.*, 2005, **127**, 8236.
22 S. Bontha, A. V. Kabanov and T. K. Bronich, *J. Control. Release*, 2006, **114**, 163.
23 Y. Kakizawa and K. Kataoka, *Adv. Drug Delivery Rev*., 2002, **54**, 203.
24 V. A. Kabanov and A. V. Kabanov, *Adv. Drug Delivery Rev*., 1998, **30**, 49.
25 I. K. Voets, A. de Keizer and M. A. Cohen Stuart, *Adv. Colloid Interface Sci*., 2009, **147–148**, 300.
26 A. V. Kabanov, *Adv. Drug Delivery Rev.*, 2006, **58**, 1597.
27 N. Lefevre, C. A. Fustin and J. F. Gohy, *Macromol. Rapid Commun.*, 2009, **30**, 1871.
28 T. K. Bronich, S. Bontha, L. S. Shlyakhtenko, L. Bromberg, T. A. Hatton and A. V. Kabanov, *J. Drug Targeting*, 2006, **14**, 357.
29 K. Prochaska, M. K. Baloch and Z. Tuzar, *Makromol. Chem*., 1979, **180**, 2521.
30 A. Guo, G. Liu and J. Tao, *Macromolecules*, 1996, **29**, 2487.
31 X. Shuai, T. Merdan, A. K. Schaper, F. Xi and T. Kissel, *Bioconjugate Chem.*, 2004, **15**, 441.
32 M. Iijima, Y. Nagasaki, T. Okada, M. Kato and K. Kataoka, *Macromolecules*, 1999, **32**, 1140.
33 K. B. Thurmond II, T. Kowalewski and K. L. Wooley, *J. Am. Chem. Soc*., 1996, **118**, 7239.
34 H. Y. Huang, T. Kowalewski, E. E. Remsen, R. Gertzmann and K. L. Wooley, *J. Am. Chem. Soc.*, 1997, **119**, 11653.
35 K. B. Thurmond II, H. Y. Huang, C. G. Jr. Clark, T. Kowalewski and K. L. Wooley, *Colloids Surf. B: Biointerfaces*, 1999, **16**, 45.
36 V. Butun, N. C. Billingham and S. P. Armes, *J. Am. Chem. Soc.*, 1998, **120**, 12135.
37 C. J. Rijcken, C. J. Snel, R. M. Schiffelers, C. F. van Nostrum and W. E. Hennink, *Biomaterials*, 2007, **28**, 5581.
38 J. O. Kim, N. V. Nukolova, H. S. Oberoi, A. V. Kabanov and T. K. Bronich, *Polym. Sci. Ser. A*, 2009, **51**, 708.
39 J. O. Kim, G. Sahay, A. V. Kabanov and T. K. Bronich, *Biomacromolecules*, 2010, **11**, 919.
40 G. Sahay, J. O. Kim, A. V. Kabanov and T. K. Bronich, *Biomaterials*, 2010, **31**, 923.
41 J. K. Oh, D. J. Siegwart, H. I. Lee, G. Sherwood, L. Peteanu, J. O. Hollinger, K. Kataoka and K. Matyjaszewski, *J. Am. Chem. Soc.*, 2007, **129**, 5939.
42 A. N. Koo, H. J. Lee, S. E. Kim, J. H. Chang, C. Park, C. Kim, J. H. Park and S. C. Lee, *Chem. Commun*., 2008, **48**, 6570.
43 Z. R. Lu, X. Wang, D. L. Parker, K. C. Goodrich and H. R. Buswell, *Bioconjugate Chem*., 2003, **14**, 715.
44 T. Ke, Y. Feng, J. Guo, D. L. Parker and Z. R. Lu, *Magn. Reson. Imaging*, 2006, **24**, 931.
45 K. Miyata, Y. Kakizawa, N. Nishiyama, A. Harada, Y. Yamasaki, H. Koyama and K. Kataoka, *J. Am. Chem. Soc*., 2004, **126**, 2355.
46 K. Miyata, Y. Kakizawa, N. Nishiyama, Y. Yamasaki, T. Watanabe, M. Kohara and K. Kataoka, *J. Control. Release*, 2005, **109**, 15.
47 Y. Kakizawa, A. Harada and K. Kataoka, *J. Am. Chem. Soc.*, 1999, **121**, 11247.
48 Y. Kakizawa, A. Harada and K. Kataoka, *Biomacromolecules*, 2001, **2**, 491.
49 S. Matsumoto, R. J. Christie, N. Nishiyama, K. Miyata, A. Ishii, M. Oba, H. Koyama, Y. Yamasaki and K. Kataoka, *Biomacromolecules*, 2009, **10**, 119.
50 M. Oba, K. Aoyagi, K. Miyata, Y. Matsumoto, K. Itaka, N. Nishiyama, Y. Yamasaki, H. Koyama and K. Kataoka, *Mol. Pharm*., 2008, **5**, 1080.
51 Y. Li, B. S. Lokitz, S. P. Armes and C. L. McCormick, *Macromolecules*, 2006, **39**, 2726.
52 T. K. Bronich, M. Ouyang, V. A. Kabanov, A. Eisenberg, F. C. Szoka and A. V. Kabanov, *J. Am. Chem. Soc*., 2002, **124**, 11872.
53 J. Zhang, Y. Zhou, Z. Zhu, Z. Ge and S. Liu, *Macromolecules*, 2008, **41**, 1444.
54 E. S. Read and S. P. Armes, *Chem. Commun.*, 2007, **29**, 3021.
55 T. G. Park, J. H. Jeong and S. W. Kim, *Adv. Drug Delivery Rev*., 2006, **58**, 467.
56 S. Mao, W. Sun and T. Kissel, *Adv. Drug Delivery Rev*., 2010, **62**, 12.
57 M. Neu, O. Germershaus, M. Behe and T. Kissel, *J. Control. Release*, 2007, **124**, 69.
58 Y. Vachutinsky, M. Oba, K. Miyata, S. Hiki, M. R. Kano, N. Nishiyama, H. Koyama, K. Miyazono and K. Kataoka, *J. Control. Release*, 2011, **149**, 51.
59 X. Jiang, Y. Zheng, H. H. Chen, K. W. Leong, T. H. Wang and H. Q. Mao, *Adv. Mater*., 2010, **22**, 1.
60 A. Harada and K. Kataoka, *Macromolecules*, 1998, **31**, 288.
61 A. Harada and K. Kataoka, *J. Control. Release*, 2001, **72**, 85.
62 X. Yuan, A. Harada, Y. Yamasaki and K. Kataoka, *Langmuir*, 2005, **21**, 2668.
63 A. Harada and K. Kataoka, *J. Am. Chem. Soc.*, 2003, **125**, 15306.
64 M. Jaturanpinyo, A. Harada, X. Yuan and K. Kataoka, *Bioconjugate Chem.*, 2004, **15**, 344.
65 N. L. Klyachko, D. S. Manickam, A. M. Brynskikh, S. V. Uglanova, S. Li, S. M. Higginbotham, T. K. Bronich, E. V. Batrakova and A. V. Kabanov, *Nanomed. Nanotechnol. Biol. Med*., 2012, **8**, 119.
66 D. S. Manickam, A. M. Brynskikh, J. L. Kopanic, P. L. Sorgen, N. L. Klyachko, E. V. Batrakova, T. K. Bronich and A. V. Kabanov, *J. Control. Release*, 2012, **162**, 636.
67 E. G. Rosenbaugh, J. W. Roat, L. Gao, R. F. Yang, D. S. Manickam, J. X. Yin, H. D. Schultz, T. K. Bronich, E. V. Batrakova, A. V. Kabanov, I. H. Zucker and M. C. Zimmerman, *Biomaterials*, 2010, **31**, 5218.
68 A. Gaydess, E. Duysen, Y. Li, V. Gilman, A. Kabanov, O. Lockridge and T. Bronich, *Chem. Biol. Interact.*, 2010, **187**, 295.
69 M. J. Heffernan and N. Murthy, *Ann. Biomed. Eng.*, 2009, **37**, 1993.

70 A. Kawamura, A. Harada, K. Kono and K. Kataoka, *Bioconjugate Chem.*, 2007, **18**, 1555.
71 M. Sotiropoulou, G. Bokias and G. Staikos, *Biomacromolecules*, 2005, **6**, 1835.
72 K. T. Oh, T. K. Bronich, V. A. Kabanov and A. V. Kabanov, *Biomacromolecules*, 2007, **8**, 490.
73 S. Lindhoud, R. Vries, W. Norde and M. A. Cohen Stuart, *Biomacromolecules*, 2007, **8**, 2219.
74 W. C. Lee, Y. C. Li and I. M. Chu, *Macromol. Biosci*., 2006, **6**, 846.
75 A. B. J. Withey, G. Chen, T. L. U. Nguyen and M. H. Stenzel, *Biomacromolecules*, 2009, **10**, 3215.
76 Y. Li, B. S. Lokitz, S. P. Armes and C. L. McCormick, *Macromolecules*, 2006, **39**, 2726
77 Y. Xu, F. Meng, R. Cheng and Z. Zhong, *Macromol. Biosci*., 2009, **9**, 1254.
78 N. Nishiyama, M. Yokoyama, T. Aoyagi, T. Okano, Y. Sakurai and K. Kataoka, *Langmuir*, 1999, **15**, 377.
79 N. Nishiyama, S. Okazaki, H. Cabral, M. Miyamoto, Y. Kato, Y. Sugiyama, K. Nishio, Y. Matsumura and K. Kataoka, *Cancer Res*., 2003, **63**, 8977.
80 H. Uchino, Y. Matsumura, T. Negishi, F. Koizumi, T. Hayashi, T. Honda, N. Nishiyama, K. Kataoka, S. Naito and T. Kakizoe, *Br. J. Cancer*, 2005, **93**, 678.
81 H. Cabral, N. Nishiyama, S. Okazaki, H. Koyama and K. Kataoka, *J. Control. Release*, 2005, **101**, 223.
82 J. O. Kim, A. V. Kabanov and T. K. Bronich, *J. Control. Release*, 2009, **138**, 197.
83 H. S. Oberoi, F. C. Laquer, L. A. Marky, A. V. Kabanov and T. K. Bronich, *J. Control. Release*, 2011, **153**, 64.
84 N. V. Nukolova, H. S. Oberoi, S. M. Cohen, A. V. Kabanov and T. K. Bronich, *Biomaterials*, 2011, **32**, 5417.
85 C. F. Van Nostrum, *Soft Matter*, 2011, **7**, 3246.

Received: 25th February 2013; Com. 13/4074