Chapter

18

DRUG-DELIVERY SYSTEMS USING MACROCYCLIC ASSEMBLIES

Yu Liu^{1,2*}, Kun-Peng Wang¹, and Yong Chen^{1,2}

¹ Department of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, P. R. China

² Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Nankai University, Tianjin 300071, P. R. China

*Corresponding author: yuliu@nankai.edu.cn

Contents

18.1. INTRODUCTION	461
18.2. CYCLODEXTRIN-BASED SUPRAMOLECULAR SYSTEMS FOR GENE DELIVERY	463
18.3. CYCLODEXTRIN-BASED SUPRAMOLECULAR SYSTEMS FOR DRUG DELIVERY	471
18.4. SULFONATOCALIXARENE-BASED NANOPARTICLES FOR DRUG DELIVERY	475
18.5. CONCLUSION	479
ACKNOWLEDGEMENT	479
REFERENCES	480

18.1. INTRODUCTION

Recently, the design of advanced drug-delivery systems with high therapeutic efficacy toward malignant tumours and insignificant toxicity to normal tissues has become one of the most challenging tasks in medicinal chemistry [1]. For an effective drug-delivery system, a high level of water solubility, controlled release, targeted delivery, biocompatibility, biodegradability and simplified delivery are all necessary. In recent decades, a variety of nano-supramolecular systems, including liposomes [2], inorganic nanoparticles [3], polymeric micelles [4] and carbon nanomaterials [5], have been constructed from multiple functional components through molecular assembly induced by host-guest complexation.

Macrocycle-based host-guest inclusion complexes consist of a host molecule with a cavity and a guest molecule inside the cavity. Generally, such a host has external features that interact with the solvent and internal features that foster binding of the guest through its specific shape and favourable environment [6]. As typical macrocyclic hosts, cyclodextrins (CDs) and sulfonatocalixarenes are non-toxic, biocompatible and have strong binding ability in water, which enables them to act as excellent platforms for drug delivery.

CDs represent a class of cyclic oligosaccharides composed of D-glucose units linked by α -1,4-glucose bonds, which are water soluble, non-toxic, commercially available compounds with a low price, and their structures are rigid and well defined. Commonly used CDs are α -, β - and γ -CDs, which are composed of 6, 7 and 8 D-glucose repeating units, respectively (Scheme 1) [7].

Scheme 1. Schematic representation of α -, β - and γ -CDs ($n = 6, 7$ or 8 , respectively)

Importantly, the three-dimensional structure of CDs can be represented as a truncated cone, with the secondary hydroxyl groups on the wider end of the cone and the primary hydroxyl groups on the smaller cone rim. This particular arrangement makes the interior of the CD cavity less hydrophilic relative to the

aqueous media and favours the hosting of hydrophobic molecules. Therefore, CDs can bind various inorganic/organic/biological molecules and ions in both aqueous solution and the solid state through a series of non-covalent interactions including hydrophobic or van der Waals interactions, the release of CD ring strain, changes in solvent-surface tensions or through hydrogen bonding with the CD hydroxyl groups [8]. The inclusion complex can alter the physical and chemical properties of the guest molecule, but typically results in the enhanced water solubility of the guest. Owing to their low price, good availability and capability of forming inclusion complexes with high water solubility, CDs are extensively studied as convenient building blocks to construct nano-structured functional materials, especially bioactive materials [9]. Since the 1970s, numerous potential applications of CDs in medicinal chemistry have been studied in terms of increasing the availability of insoluble substrates, reducing substrate inhibition, limiting product inhibition and as delivery agents. For example, CDs are very useful for solubilising drugs and their carriers. In our previous work, we found that the water solubility of paclitaxel could be increased to 2 mg mL^{-1} in a supramolecular assembly formed of tetraethylenepentaamino-bridged bis(β -CD) and two paclitaxel complexes [10].

Scheme 2. Schematic representation of the SCnAs ($n = 4-8$) family

Another typical macrocyclic host is the *p*-sulfonatocalix[*n*]arene (SCnA, $n = 4-8$) family of water-soluble calixarene derivatives that bind guest molecules to their cavities in aqueous media (Scheme 2) [11]. SCnAs possess three-dimensional and π -electron-rich cavities with multiple sulfonate groups, which endow them with fascinating affinities and selectivities, especially toward organic cations [12]. They can also serve as scaffolds for functional and responsive

host-guest systems [13]. Moreover, SCnAs are biocompatible, which makes them potentially useful for diverse life-science and pharmaceutical applications. In this part, we highlight some typical nano-structured assemblies based on CDs and SCnAs as well as their important applications in drug delivery.

18.2. CYCLODEXTRIN-BASED SUPRAMOLECULAR SYSTEMS FOR GENE DELIVERY

Gene therapy is the use of genes (DNA and RNA) instead of conventional drugs to treat diseases. Gene therapy has drawn more and more attention in recent years as a potential means of treatment. Considering that naked genes are not effectively endocytosed by cells and are easily degraded by serum nucleases [14], it is important for scientists to find efficient and safe gene delivery systems [15]. Different from viral carriers, non-viral carriers have low immunogenicity, good biocompatibility and satisfactory DNA-loading capability. Therefore, non-viral gene carriers such as liposomes, polymers and dendrimers have been widely used as vectors for gene therapy. Recently, CD-based supramolecular systems have attracted great attention for gene delivery, because CDs can bind with nucleic acids and increase their stability against nuclease as well as improve cellular uptake. For example, the controlled condensation of DNA is one of the key steps in gene delivery and gene therapy [16]. Recently, to increase transfection efficiency and decrease toxicity, cationic CDs and CD-modified polycations have been used as novel vectors for gene delivery [17].

CD-based polypseudorotaxanes are a type of supramolecular assembly with CDs threading onto the polymer chains, and they are stabilised by hydrogen bonding between adjacent CD cavities as well as through non-covalent interactions between the long-chain molecule and the threaded CD cavities [18]. Interestingly, CD-based polypseudorotaxanes can be converted to CD-based polyrotaxanes by introducing bulky terminals (bulky organic or organometallic groups) at the chain ends in order to prevent the de-threading of CDs. These bioactive CD-based polypseudorotaxanes or polyrotaxanes constructed by threading CDs with polycations and/or fused-ring aromatic substituents onto the polymer chain have widely been used to interact with DNA.

As a good bioactive precursor, anthryl-modified CDs are good chemically switched DNA intercalation materials [19]. By threading anthryl-modified β -CDs onto the poly(propylene glycol) bis(2-aminopropyl ether) (PPG-NH₂, molecular weight (*MW*) = 2000 Da) chains, polypseudorotaxanes bearing several anthryl groups can be obtained easily with an average of ten CD units per PPG chain [20]. This polypseudorotaxane can condense the originally loose, free DNA into solid particles with an average diameter of approximately 100 nm (Figure 1), as demonstrated by fluorescence titration and atomic force microscopy (AFM) (Figure 2). From molecular modelling studies, one can find that

anthryl-modified β -CDs in polypseudorotaxane can intercalate both the minor and major DNA grooves. Therefore, the driving force of DNA condensation should not only be the electrostatic interactions between the protonated amino groups in polypseudorotaxane and the negatively charged phosphates

in DNA, but also the intercalation of multiple anthryl groups in the DNA grooves.

Figure 1. Structure of anthryl-modified CD-based polypseudorotaxanes

Figure 2. AFM images of (a) free calf-thymus DNA and (b) condensed DNA induced by CD-based polypseudorotaxane with anthryl grafts

Another typical example is a two-dimensional cationic polypseudorotaxane constructed by threading 6-[(6-amino-6-deoxy- β -CD)amino] dichloride molecules onto the polymer backbone, followed by complexing cucurbit[6]uril

(CB[6]) units on the branches of the modified CDs (Figure 3) [21]. Owing to the strong binding between hexane-1,6-diamine and CB[6] ($K = 4.49 \times 10^8 \text{ M}^{-1}$) in aqueous solution [22], the degree of CB[6] substitution can be controlled. This two-dimensional cationic polypseudorotaxane displays controllable DNA condensation ability by adjusting the amount of CB[6] in the polypseudorotaxane, as CB[6] on spermidine and spermine affects their ability to adjust the activity of a DNA enzyme. The DNA condensation ability of this polypseudorotaxane reaches its highest efficiency with 70 % CB[6]. Further investigations using agarose-gel electrophoresis, ethidium-bromide displacement and AFM experiments demonstrate that the effective charges of polypseudorotaxane interacting with DNA and the changing rigidity of polypseudorotaxane with the addition of CB[6] jointly lead to the unusual DNA condensation ability of the two-dimensional polypseudorotaxane.

Figure 3. Structure of the two-dimensional polypseudorotaxane

CD-based polyrotaxanes are also used in gene delivery. Cationic CD-containing polyrotaxanes constructed by threading cationic CD derivatives onto polymer backbones show good DNA-binding ability, low cytotoxicity and high gene-transfection efficacy [23]. For example, a type of polyrotaxane constructed from oligoethylenimine-grafted β -CDs threading onto the polymer chain, which possesses a high cation density, shows high gene-transfection efficiency with and without serum. Moreover, the transfection efficiency of these cationic polyrotaxanes, in most cases, increases with elongation of oligoethylenimine grafts on the β -CD units.

Through the strong Au-S binding, thio- or polythio-modified CDs can be absorbed on the surface of gold to form three-dimensional supramolecular

assemblies. In a typical example, a supramolecular assembly is constructed by adsorbing oligo(ethylenediamino)-CDs on gold nanoparticles (Figure 4) [24]. Possessing many CD cavities at the outer space, this assembly is demonstrated to be a good vector for DNA binding as well as having moderate plasmid transfection efficiency as a carrier in cultivated cells *in vitro*, which are sufficiently investigated by means of circular-dichroism spectroscopy, transmission electron microscopy and visual GFP expression.

Figure 4. Oligo(ethylenediamino)-CD-modified gold nanoparticles (AuNPs)

By further introducing anthryl adamantanes into the supramolecular assembly containing AuNPs and β -CDs, the obtained secondary supramolecular assembly exhibits good condensation ability toward calf thymus DNA (ct-DNA) owing to the good DNA reactivity with anthracene moieties (Figure 5) [25]. AFM images show that, with an increasing guest-to-host ratio, more and larger aggregates are formed (Figure 6), demonstrating that the CD–AuNP/anthryl adamantane system can act as a promising DNA concentrator and give good binding abilities toward ct-DNA. In addition, the condensation efficiency can be conveniently controlled by adjusting the ratio between the AuNPs and anthryl adamantane grafts. The larger size of the DNA supramolecular aggregates is beneficial to their intracellular uptake, and the smaller size of free complexes of CD-modified AuNPs/anthryl adamantane means that the complexes can be eliminated from the cell more quickly after completion of the delivery mission. Therefore, this supramolecular nanostructure may have exciting applications in gene therapy with the promising potential to control gene expression and delivery.

Figure 5. Construction of the CD-AuNP/anthryl adamantine host-guest system

Figure 6. AFM images of ct-DNA (0.37 g L^{-1}) in the absence (a) and presence of CD-modified AuNPs/anthryl adamantine with different guest/host ratios: 1/20 (b), 1/5 (c) and 1/1 (d)

In addition, the amino-terminated polypseudorotaxane can also attach to the surface of the AuNPs to form three-dimensional nanocages through electrostatic interactions between the amino terminals of polypseudorotaxane and the gold nuclei (Figure 7). Interestingly, this type of nanocage constructed by the attachment of numerous L-Try-CD-based polypseudorotaxanes onto the surface of the AuNP only gives weak DNA cleavage ability [26]. However, after being saturated with buckminsterfullerene (C_{60}), the nanocage exhibits a much

higher DNA cleavage activity under visible-light irradiation, and most of the closed supercoiled DNA strands are cleaved to form nicked circular DNA [27].

Figure 7. Structure of L-Try-CD-based polypseudorotaxane

In addition to gold particles, carbon nanotubes are also used as templates to construct three-dimensional CD-based supramolecular assemblies. Many linear macromolecules, including organic polymers and biomacromolecules, are able to couple with carbon nanotubes through non-covalent wrapping or adsorption. Therefore, nanotube/CD supramolecular assemblies can be constructed conveniently by wrapping or adsorbing CD-polymers on the carbon nanotubes.

As the surface of the nanotube is hydrophobic, it hardly interacts with the double-stranded DNA, where the hydrophilic sites (phosphates) are exposed on the surface. However, after wrapping an anthryl CD-based polypseudorotaxane on the surface of a carbon nanotube, the resultant nanotube/polypseudorotaxane supramolecular assembly shows good ability in terms of wrapping and cleaving double-stranded DNA (Figure 8) [28]. The adsorption of CDs onto the carbon nanotube and the intercalation of anthryl groups into the DNA grooves may play important roles in DNA wrapping.

Figure 8. Schematic representation of DNA wrapping for a nanotube/polypseudorotaxane supramolecular assembly

In another typical example, a β -CD-modified chitosan moiety shows moderate DNA condensation ability and is able to condense free DNA to form uniform hollow loops [29]. After associating adamantanyl pyrene molecules to β -CD-modified chitosan, the dyad with exposed pyrene grafts is more effective in condensing DNA than β -CD-modified chitosan, and the free DNA strands are condensed to solid particles with an average diameter of approximately 200 nm by the dyad, rather than forming hollow loops by the β -CD-modified chitosan (Figure 9). The enhancement in the DNA condensing efficiency is ascribed to the cooperative contribution of aromatic pyrenes and inherent ammonium cations on the chitosan surface. Interestingly, by wrapping β -CD-modified chitosan on the carbon nanotube, the resultant dyad can condense free DNA to compact particles with an average diameter of approximately 80 nm. The wrapping of β -CD-modified chitosan rearranges the β -CD-modified chitosan on the surface of the carbon nanotube into highly dispersed polymers, which enables more active ammonium cation interactions with DNA grooves. Inspired by the improved DNA condensation shown by chitosan/pyrene and nanotube/chitosan dyads, a nanotube/chitosan/pyrene triad is tested as a combinatorial vector, which shows a promoted DNA condensation ability compared with that of the nanotube/chitosan dyad.

Figure 9. AFM images (a-d) of DNA condensation induced by CD-modified chitosan, chitosan/pyrene, nanotube/chitosan or nanotube/chitosan/pyrene dyads

Figure 10. Structure of PEI-Ada-LCD@DNA assemblies

Poly(ethyleneimine) (PEI, 25 kDa), one of the most effective gene-delivery vectors studied to date, has a high buffer capacity that can protect DNA from the degradation of nuclease, but it also induces higher toxicity in the biological process on account of their non-biodegradability. To construct safe and

effective gene-delivery carriers, a CD-based cross-linking system of low-molecular-weight PEI is designed as an effective way to reduce the cytotoxicity at a high gene expression level (Figure 10) [30]. Herein, the supramolecular cross-linking system, composed of adamantyl-modified PEI and L-cystine-bridged bis(β -CD), is used as a bioavailable recycling DNA carrier through host-guest interactions, and shows better DNA condensation, transfection ability and lower cytotoxicity than 25 kDa PEI. Significantly, the disulfide bond in the cross-linking sites can be cleaved easily by reductive enzymes to promote DNA release. This assembly not only avoids the complicated synthesis/separation steps that are always involved in covalently modifying PEI, but also provides a non-viral gene carrier with stronger gene condensation affinity, higher gene transfection efficiency and lower cellular toxicity, which will energise the potential use of CD-based bioactive supramolecular nanostructures in the construction of safe and highly efficient gene carriers.

18.3. CYCLODEXTRIN-BASED SUPRAMOLECULAR SYSTEMS FOR DRUG DELIVERY

In recent years, the construction of carrier-mediated artificial systems through a supramolecular methodology has offered a powerful strategy to design and construct drug formulations and delivery agents [31]. CDs possess well-recognised biocompatibility and ability to form stable complexes, making them attractive as building blocks for the construction of nano-scale functional and bioactive materials [32]. CD-based materials, including amphiphilic CDs, CD-polymers, CD-pendant polymers and CD-based polyrotaxanes, can form nano-structured assemblies such as micelles, nano-gels and vesicles, which exhibit multiple hydrophilic/hydrophobic domains and recognition sites, and, therefore, are potential nanocarriers for both hydrophilic and hydrophobic bioactive molecules [33].

In order to construct versatile nano-assembled drug carriers, a supramolecular assembly of folic acid (FA)-modified β -CD and graphene oxide (GO) non-covalently linked by an adamantane-grafted porphyrin is constructed [34]. Herein, GO, with a thickness of one atom and a large two-dimensional structure, can strongly bind to various organic or biological molecules through chemical modifications, thus promoting practical innovations in biological systems [35], such as nanometre-sized carriers of drugs and genes. Benefiting from strong π - π stacking between the porphyrin and GO and the high hydrophobic affinity of CD for adamantane, the resulting quaternary supramolecular nanoarchitecture can be employed as a delivery platform to efficiently carry doxorubicin hydrochloride (DOX) (Figure 11). Owing to the targeting effect of FA, the concentration of the assembly in normal tissues may remain at lower level, thereby reducing toxicity to normal cells. Therefore, this

system may represent a general protocol for a large library of multifunctional supramolecular biomaterials and can provide a new potential pathway to comprehensively understand the applicability of bioactive, nanoscale materials.

Figure 11. Complex of GO, DOX, adamantane-modified porphyrin and FA-modified CD

Furthermore, hydrophobic camptothecin (CPT) can also be delivered using a CD-based system [36]. As the hyaluronic acid (HA) skeleton can specifically recognise various cancer cells that over-express HA receptors on the cell surface [37], hyaluronated adamantine (HA-ADA) is chosen as the target molecule, whereas CD-modified GO (GO-CD) is used as a scaffold (Figure 12). Benefiting from the supramolecular complexation of the β -CD cavity with the adamantyl group and the π - π stacking interaction between the planar GO surface and the drug molecule, a ternary assembly of CPT@GO-CD/HA-ADA is successfully constructed, and CPT is successfully endowed with water solubility. The inclusion complex of CD/ADA prevents the GO skeletons from intermolecular aggregation in water, which then facilitates the disruption of GO sheets into small-sized components. In the cytotoxicity experiments, CPT@GO-CD/HA-ADA exhibits a higher curative effect and a lower

cytotoxicity than a free drug. The conventional chemotherapeutic drugs can be specifically delivered to their intended sites of action, and their general toxicity can be reduced, to a significant extent, for wider clinical applications.

Figure 12. Structure of the CPT@GO-CD/HA-ADA supramolecular assembly

Based on the target ability of HA, new conjugated polysaccharides: CD-grafted hyaluronic acid (HACD) composed of an HA main chain and CD side chains are constructed. Then, HACD and the adamplatins prodrug can form hydroxyapatite (HAP) nanoparticles that possess a hydrophilic HA backbone for recognising cancer cells as a delivery system for an adamplatins prodrug both *in vitro* and *in vivo* (Figure 13) [38]. The anti-tumour activity of HAP in mice is comparable to the commercial anticancer drug cisplatin, but the toxicity to normal cells is much lower. The specific binding of HA on the backbone of HAP to the HA receptors that are over-expressed on tumour cells not only allows receptor-mediated endocytosis of HAP into tumour cells and tissues, but also prevents normal cells and tissues from being damaged. The present methodology provides a versatile HA platform for targeted drug delivery and transport into cancer cells, while exhibiting minimal uptake into normal tissues.

Figure 13. Structure of conjugated HAP

Figure 14. Schematic illustration of the chemical structures of the HACD-AuNPs

Through the high affinity of the β -CD cavity for adamantane moieties, polysaccharide-gold nanocluster supramolecular conjugates (HACD-AuNPs), which consist of AuNPs bearing adamantane moieties and HACD, can also be constructed as the delivery platform (Figure 14) [39]. Owing to their porous

structure, this supramolecular conjugate can serve as a versatile and biocompatible platform for the loading and delivery of various anticancer drugs, such as DOX, paclitaxel (PTX), camptothecin (CPT), irinotecan hydrochloride (CPT-11) and topotecan hydrochloride (TPT). DOX encapsulation and its loading efficiency are calculated to be 78.68 and 11.03 %, respectively. Significantly, the DOX@HACD-AuNPs displays slow and controlled release of the drug, with the release rate measured to be 3–4 times lower than that of free DOX in acidic or neutral environments. Owing to the high efficiency of their cellular uptake by HA-reporter-mediated endocytosis, the resulting drug@HACD-AuNP system effectively inhibits the growth of MCF-7 cells, enables pH-responsive drug release in cells and decreases drug toxicity toward normal cells. Furthermore, this carrier can provide new possibilities in the development of targeted drug delivery and biomedical applications.

18.4. SULFONATOCALIXARENE-BASED NANOPARTICLES FOR DRUG DELIVERY

Different to CDs, SCnAs can promote the self-aggregation of aromatic and amphiphilic molecules by lowering the critical aggregation concentration (CAC), enhancing aggregate stability and compactness as well as regulating the degree of order in the aggregates. This unique self-assembly strategy has been defined as calixarene-induced aggregation (CIA) [40]. The number of guest species has been divided into four categories: aromatic fluorescent dyes [41], amphiphilic surfactants [42], drugs [43] and proteins [44].

Owing to the biocompatibility of SCnAs, a lot of research on the use of CIA is devoted toward fabricating supra-amphiphiles, which are of fundamental interest for drug-delivery applications. For example, *p*-sulfonatocalix[5]arene (SC5A) as the host and 1-pyrenemethylaminium (PMA) as the guest were first used to fabricate self-assembled binary supramolecular vesicles (Figure 15), which can successfully load DOX [45]. This amphiphilic self-assembly has an average diameter of 99 nm and a narrow size distribution according to a dynamic laser-scattering experiment. Transmission electron microscopy (TEM) shows a hollow spherical morphology, convincingly indicating a vesicular structure. The thickness of the bilayer membrane is about 3 nm, which is on the same order of magnitude as the sum of one PMA length (7 Å) and two SC5A heights (14 Å), indicating that the vesicle is unilamellar. From the obtained details, the model of supramolecular vesicles can be deduced to have hydrophobic pyrene segments packed together, with inner- and outer-layer surfaces consisting of hydrophilic phenolic hydroxyl groups of SC5A, which are exposed to water. SC5A and PMA are connected together by host-guest and charge interactions. After purification by ultracentrifugation and dialysis, DOX is successfully loaded into the vesicle. The loaded DOX molecules

can be released upon warming, together with the disassembly of the vesicles, as proven by detecting the amplification of the fluorescence signal of DOX that is accompanied with a temperature increase. Excess SC5A leads to the formation of a 1 : 1 inclusion complex, accompanied by the disassembly of the amphiphilic aggregation.

Figure 15. Construction of the SC5A+PMA supramolecular binary vesicles and temperature-responsive drug release from the vesicles

Many biomacromolecules, such as proteins and nucleic acids, change their behaviour in response to a combination of environmental stimuli, rather than to a single stimulus. The construction of materials that can mimic this feature is of great interest. Therefore, multi-stimuli-responsive supramolecular vesicles are constructed through the CIA theory of *p*-sulfonatocalix[4]arene (SC4A) (Figure 16) [46]. The resulting vesicles respond to multiple stimuli, including temperature, the addition of CD and redox reactions, benefiting from the intrinsic advantages of supramolecular species. The architecture of these vesicles that contain entrapped DOX can be disrupted through the reduction of viologen to its neutral form, by increasing the temperature or upon the addition of CDs; the disruption triggers the efficient release of the entrapped DOX from the vesicle interior. During *in vitro* experiments, the loading of DOX into the vesicles does not affect its toxicity to cancer cells, whereas encapsulation reduces damage to normal cells.

Figure 16. Formation of a multi-stimulus-responsive supramolecular binary vesicle composed of SC4A and an asymmetric viologen

Amphiphilic self-assemblies that respond to enzymatic reactions represent an increasingly important topic in biomaterial research, and applications of such assemblies for the controlled release of therapeutic agents at specific sites where a target enzyme is located are feasible. For example, cholinesterase-responsive supramolecular vesicles based on SC4A and myristoylcholine are used as a targeted drug-delivery system (Figure 17) [47]. Amphiphilic myristoylcholine cannot be used alone to fabricate an enzyme-responsive assembly, because the CACs of the substrate (myristoylcholine) and the product (choline) are similar. Complexation of SC4A with myristoylcholine directs a supramolecular binary vesicle and decreases the CAC of myristoylcholine by a factor of approximately 100. As the components are held together by non-covalent interactions, the assembled and unassembled states are in dynamic equilibrium, and the enzymatic cleavage of free myristoyl chloride results in the disintegration of the self-assembled vesicles. The binary vesicles consisting of SC4A and myristoylcholine respond specifically and efficiently to cholinesterase, and the cholinesterase-induced cleavage of myristoylcholine disrupts the hydrophilic–hydrophobic balance of the binary super-amphiphiles, resulting in vesicle disassembly. In addition, the release of

a drug, such as the Alzheimer's drug tacrine, encapsulated in the vesicles can be triggered by this enzymatic cleavage. Cholinesterase is over-expressed in Alzheimer's disease and, therefore, this system has potential utility in the delivery of Alzheimer's drugs [48].

Figure 17. Enzymatic responsive of amphiphilic assemblies of myristoylcholine fabricated in the presence of SC4A

More recently, a trypsin-responsive supramolecular vesicle was further fabricated by employing SC4A as the macrocyclic host and protamine as the enzyme-cleavable guest. Differing from the small-molecule species employed in CIA previously, the protamine guest is a non-amphiphilic natural biological cationic protein, which greatly expanded the range of engaging substrates in fabricating CIA assemblies. The obtained vesicle is conceptually applicable as a controllable-release model at over-expressed trypsin sites. Prospectively, this proof-of-concept is adaptive to build various enzyme-triggered self-assembled materials as smart controlled-release systems that are capable of a site-specific response.

18.5. CONCLUSION

Nano-scaled supramolecular systems have been considered the most promising carriers for drug and gene delivery. With delicate design and well-controlled manipulation, nano-supramolecular systems possess a variety of functions, such as prolonged circulation, broad loading spectrum, suitable size and shape for tissue penetration and passive targeting, which are easy to tailor for active targeting at different levels and controllable release.

In recent decades, supramolecular assemblies constructed from CDs and SCnAs for drug and gene delivery have increasingly attracted the attention of chemical and biological scientists. The past two decades have witnessed a significant harvest in CD-based bioactive supramolecular assemblies. The future of CD-based supramolecular systems in drug and gene delivery is promising, in view of the notable clinical success of new pharmaceuticals based on parent CDs, their small-molecule derivatives and CD-containing polymers as well as other controlled delivery systems. In the next section, we highlight various stimulus-responsive vesicles based on CIA theory for drug delivery. These results demonstrate the feasibility of using SCnAs in disease therapy.

Finding methods to utilise host-guest interactions is a challenge, and such methods can be expected to permit the establishment of novel strategies for molecular recognition, sensing and assembly. In the future, more exciting findings and the potential of macrocyclic supramolecular assemblies are going to be discovered.

ACKNOWLEDGEMENT

We thank the 973 Program (2011CB932502) and NNSFC (91227107, 21432004, 21272125) for financial support.

REFERENCES

1. M.E. Caldorera-Moore, W.B. Liechty, N. Peppas. *Acc. Chem. Res.* **44** (2011) 1061–1070.
2. M.A. Mintzer, E.E. Simanek. *Chem. Rev.* **109** (2009) 259–302.
3. M. Vallet-Regi, M. Colilla, B. Gonzalez. *Chem. Soc. Rev.* **40** (2011) 596–607.
4. P. Tanner, P. Baumann, R. Enea, O. Onaca, C. Palivan, W. Meier. *Acc. Chem. Res.* **44** (2011) 1039–1049.
5. X. Sun, Z. Liu, K. Welsher, J.T. Robinson, A. Goodwin, S. Zaric, H. Dai. *Nano Res.* **1** (2008) 203–212.
6. E.A. Appel, J. del Barrio, X.J. Loh, O.A. Scherman. *Chem. Soc. Rev.* **41** (2012) 6195–6214.
7. J. Szejtli. *Chem. Rev.* **98** (1998) 1743–1753.
8. M.V. Rekharsky, Y. Inoue. *Chem. Rev.* **98** (1998) 1875–1917.
9. M.E. Davis, M.E. Brewster. *Nat. Rev. Drug Discov.* **3** (2004) 1023–1035.
10. Y. Liu, G.-S. Chen, L. Li, H.-Y. Zhang, D.-X. Cao, Y.-J. Yuan. *J. Med. Chem.* **46** (2003) 4634–4637.
11. D.-S. Guo, Y. Liu. *Chem. Soc. Rev.* **41** (2012) 5907–5921.
12. K.-P. Wang, D.-S. Guo, H.-X. Zhao, Y. Liu. *Chem. Eur. J.* **20** (2014) 4023–4031.
13. K.-P. Wang, Y. Chen, Y. Liu. *Chem. Commun.* **51** (2015) 1647–1649.
14. R. Niven, R. Pearlman, T. Wedeking, J. Mackeigan, P. Noker, L. Simpson-Herren, J.G. Smith. *J. Pharm. Sci.* **87** (1998) 1292–1299.
15. C. Ortiz Mellet, J.M. García Fernández, J.M. Benito. *Chem. Soc. Rev.* **40** (2011) 1586–1608.
16. S. Angelos, N.M. Khashab, Y.-W. Yang, A. Trabolsi, H.A. Khatib, J.F. Stoddart, J.I. Zink. *J. Am. Chem. Soc.* **131** (2009) 12912–12914.
17. S. Srinivasachari, T.M. Reineke. *Biomaterials* **30** (2009) 928–938.
18. A. Harada. *Acc. Chem. Res.* **34** (2001) 456–464.
19. T. Ikeda, K. Yoshida, H.-J. Schneider. *J. Am. Chem. Soc.* **117** (1995) 1453–1454.
20. Y. Liu, L. Yu, Y. Chen, Y.-L. Zhao, H. Yang. *J. Am. Chem. Soc.* **129** (2007) 10656–10657.
21. C.-F. Ke, S. Hou, H.-Y. Zhang, Y. Liu, K. Yang, X.-Z. Feng. *Chem. Commun.* (2007) 3374–3376.
22. S. Liu, C. Ruspic, P. Mukhopadhyay, S. Chakrabarti, P.Y. Zavalij, L. Isaacs. *J. Am. Chem. Soc.* **127** (2005) 15959–15967.
23. J. Li, X.J. Loh. *Adv. Drug Deliv. Rev.* **60** (2008) 1000–1017.
24. H. Wang, Y. Chen, X.-Y. Li, Y. Liu. *Mol. Pharma.* **4** (2007) 189–198.
25. D. Zhao, Y. Chen, Y. Liu. *Chem. Asian J.* **9** (2014) 1895–1903.
26. Y. Liu, H. Wang, Y. Chen, C.-F. Ke, M. Liu. *J. Am. Chem. Soc.* **127** (2005) 657–666.
27. Y. Liu, Y.-L. Zhao, Y. Chen, M. Wang. *Macromol. Rapid Commun.* **26** (2005) 401–406.
28. Y. Chen, L. Yu, X.-Z. Feng, S. Hou, Y. Liu. *Chem. Commun.* **27** (2009) 4106–4108.
29. Y. Liu, Z.-L. Yu, Y.-M. Zhang, D.-S. Guo, Y.-P. Liu. *J. Am. Chem. Soc.* **130** (2008) 10431–10439.
30. Y.-H. Zhang, Y. Chen, Y.-M. Zhang, Y. Yang, J.-T. Chen, Y. Liu. *Sci. Rep.* **4** (2014) 7471.
31. D. Peer, J.M. Karp, S. Hong, O.C. Farokhzad, R. Margalit, R. Langer. *Nat. Nanotechnol.* **2** (2007) 751–760.
32. Y. Chen, Y.-M. Zhang, Y. Liu. *Chem. Commun.* **46** (2010) 5622–5633.

33. J. Zhang, P.X. Ma. *Adv. Drug Deliv. Rev.* **65** (2013) 1215–1233.
34. Y. Yang, Y.-M. Zhang, Y. Chen, D. Zhao, J.-T. Chen, Y. Liu. *Chem. Eur. J.* **18** (2012) 4208–4215.
35. Z. Liu, W.B. Cai, L.N. He, N. Nakayama, K. Chen, X.M. Sun, X.Y. Chen, H.J. Dai. *Nat. Nanotechnol.* **2** (2007) 47–52.
36. Y.-M. Zhang, Y. Cao, Y. Yang, J.-T. Chen, Y. Liu. *Chem. Commun.* **50** (2014) 13066–13069.
37. S.-Y. Han, H.S. Han, S.C. Lee, Y.M. Kang, I.-S. Kim, J.H. Park. *J. Mater. Chem.* **21** (2011) 7996–8001.
38. Y. Yang, Y.-M. Zhang, Y. Chen, J.-T. Chen, Y. Liu. *J. Med. Chem.* **56** (2013) 9725–9736.
39. N. Li, Y. Chen, Y.-M. Zhang, Y. Yang, Y. Su, J.-T. Chen, Y. Liu. *Sci. Rep.* **4** (2014) 4164.
40. D.-S. Guo, Y. Liu. *Acc. Chem. Res.* **47** (2014) 1925–1934.
41. D.-S. Guo, B.-P. Jiang, X. Wang, Y. Liu. *Org. Biomol. Chem.* **10** (2012) 720–723.
42. Y. Cao, Y.-X. Wang, D.-S. Guo, Y. Liu. *Sci. China Chem.* **57** (2014) 371–378.
43. Z. Qin, D.-S. Guo, X.-N. Gao, Y. Liu. *Soft Matter* **10** (2014) 2253–2263.
44. K. Wang, D.-S. Guo, M.-Y. Zhao, Y. Liu. *Chem. Eur. J.* **20** (2014) 1–10.
45. K. Wang, D.-S. Guo, Y. Liu. *Chem. Eur. J.* **16** (2010) 8006–8011.
46. K. Wang, D.-S. Guo, X. Wang, Y. Liu. *ACS Nano* **5** (2011) 2880–2894.
47. D.-S. Guo, K. Wang, Y.-X. Wang, Y. Liu. *J. Am. Chem. Soc.* **134** (2012) 10244–10250.
48. D.-S. Guo, T.-X. Zhang, Y.-X. Wang, Y. Liu, *Chem. Commun.* **49** (2013) 6779–6781.