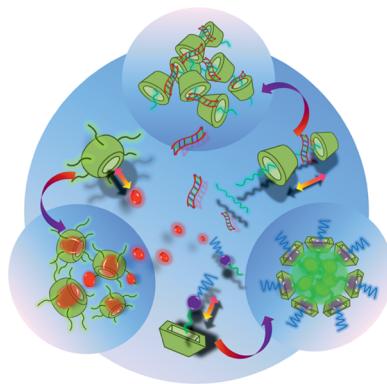


Biomedical Applications of Supramolecular Systems Based on Host–Guest Interactions

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1. HOST–GUEST SUPRAMOLECULAR CHEMISTRY

The development of supramolecular chemistry dates back to 1987 when Lehn, Cram, and Pedersen won the Nobel Prize on account of their leading discoveries in the host–guest systems.¹ Ever since its discovery, the concept of supramolecular chemistry has attracted lots of attention from chemists, biologists, and material scientists, where they utilize the noncovalent interactions, including hydrogen-bonding interaction, π – π stacking interaction, electrostatic interaction, van der Waals force, and hydrophobic/hydrophilic attraction, to explain the systems from easy to complicated.^{2–6} During the past decades, considerable efforts have been paid to develop numerous supramolecular systems and to investigate their applications in catalysis, functional materials, electronic devices, sensors, nanomedicine, and so on.^{7–11}

1.1. Introduction

As compared to covalent interactions, the noncovalent interactions present several advantages. First, the noncovalent interactions provide easy and facile approaches for building supramolecular structures, which could avoid multiple synthesis steps and a complicated purification process during the fabrications.¹² Such supramolecular methods are often cost-effective and environmentally friendly. By mixing the building blocks in solution at ambient conditions, supramolecular materials can be formed through intrinsic self-assembly. Supramolecular materials have a broad definition, which cover any materials consisting of components connected by noncovalent interactions and experiencing spontaneous assembly or disassembly processes.^{13–16} Because of the noncovalent linkage by relatively weak and dynamic interactions, supramolecular materials then have the reversibility, allowing convenient dissociation and reconstruction of the supramolecular systems at a low energy cost.¹⁷ Thus, the supramolecular materials are capable of being recycled and self-repaired from external mechanical damage. Furthermore, it has adaptive capability in response to external stimuli that can trigger the structure change of supramolecular materials.¹² Upon external stimuli, the supramolecular materials can rearrange their structures or morphologies toward most stable states driven by the decrease of Gibbs free energy. Therefore, this adaptive capability can be utilized for the design and fabrication of stimuli-responsive functional materials based on supramolecular chemistry,^{18–22} which were applied in various fields, such as fluorescent sensing²³

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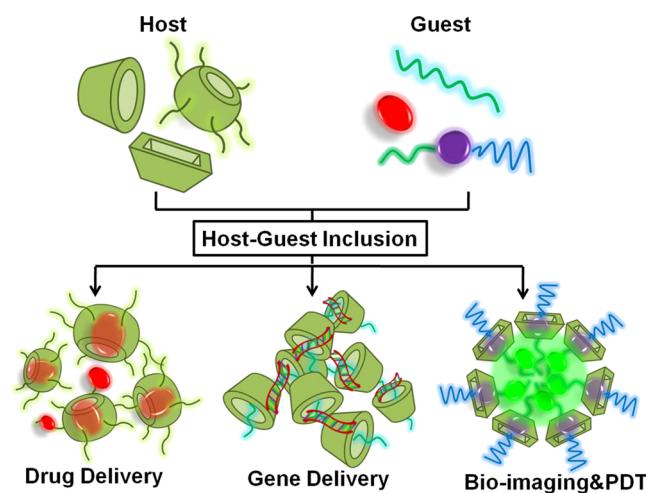
and responsive polymer gels.^{24–27} At last, supramolecular chemistry provides a possibility of manipulating molecules or supramolecular building blocks at a molecular level, allowing the “bottom-up” method to control the sizes and morphologies of the resulting supramolecular materials. Especially, the fabrication of supramolecular materials with uniform size within nanometer range has become a hot research topic, because nanomedicine presents promising potentials for revolutionizing traditional biomedical techniques. Building supramolecular systems will provide a variety of novel diagnostic and therapeutic platforms toward applications in nanomedicine.²⁸

Because of the above-mentioned advantages along with good biocompatibility or low toxicity of certain molecules and materials used, supramolecular systems have been widely utilized in the biological field.^{29–34} For example, supramolecular self-assembly of biological building blocks in water was discussed by Zayed and coauthors in their review article.³⁰ Yoon and Jang reviewed polymeric supramolecular systems developed for drug delivery, which include polymeric micelles, vesicles, and polymeric hydrogels.³⁴ Zhao and coauthors summarized the recent research progress of self-assembly systems for the fabrication and application of bioinspired materials from the view of biomimetic chemistry.³²

Among various noncovalent interactions under the definition of supramolecular chemistry, host–guest interaction based on macrocyclic molecules is a very important phenomenon that has been extensively investigated. Through such host–guest inclusion, two or more chemical moieties can be integrated together in a facile and reversible manner, providing vast possibilities for the construction of novel supramolecular structures. During the past few decades, a series of macrocyclic molecules and their derivatives have been developed, including calixarenes (CAs), crown ethers, cyclodextrins (CDs), cyclophanes, cucurbit[n]urils (CBs), pillar[n]arenes, and so on. These macrocyclic molecules are regarded as the hosts, possessing the cavities to encapsulate the guests. Usually, external property of the host molecules favors the interaction with surrounding solvent, while the internal features of their cavities facilitate the guest inclusion via hydrophobic interactions, hydrogen-bonding interactions, electrostatic interaction, specific molecular shape or size matching, etc. The most common case is to encapsulate hydrophobic guest molecules into hydrophobic cavities of macrocyclic molecules in aqueous solution. Such host–guest inclusion has relatively high stability, providing reliable and robust connection for the fabrication of supramolecular systems. For instance, the binding constant (K) for CD-based host–guest complexes can reach 10^4 M^{-1} , and that of CB-based host–guest complexes can be as high as 10^{15} M^{-1} .³³ Among these macrocyclic molecules, CAs, CDs, and CBs have attracted an increasing popularity, especially for their applications in biomedical field. One major reason is that these macrocyclic molecules are basically friendly to the biological environment and exhibit good biocompatibilities.^{35–39} Another reason is that the host–guest complex formation based on these macrocyclic molecules is a facile and reversible process, which provides the feasibilities to design stimuli-responsive supramolecular systems.¹⁸

Taking account of a large amount of scientific publications regarding the biological applications of supramolecular chemistry, this Review mainly discusses the fabrication and biomedical applications of supramolecular systems based on host–guest interactions (Scheme 1). This interesting topic has gained a lot of attention on account of the specificity and versatility of the host–

Scheme 1. Biomedical Applications of Supramolecular Systems Based on Host–Guest Interactions



guest interactions. This Review covers three major macrocyclic host molecules, including CAs, CDs, and CBs. The specific biomedical applications of the host–guest systems discussed contain several leading directions, that is, drug delivery, gene delivery, drug/gene codelivery, bioimaging, and photodynamic therapy (PDT).

1.2. Macrocyclic Host Molecules

In a typical host–guest inclusion complex, a host molecule affords a cavity to encapsulate a guest molecule through noncovalent interactions. Because of the significant role of the macrocyclic molecules in the host–guest systems, the physical property, chemical nature, and related biological activity of the macrocyclic molecules are first introduced in this Review. Three major host molecules including CAs, CDs, and CBs are particularly discussed on account of their wide applications in the biomedical field (Figure 1).

1.2.1. Cyclodextrins (CDs). Among the many host molecules discovered and utilized for building up supramolecular systems, CDs are a popular class of macrocyclic rings that have attracted a lot of attention, especially for their biological applications. CDs refer to a series of macrocyclic molecules composed of α -1,4 glycosidic bond linked oligosaccharides, which can be produced from enzyme triggered starch degradation. They were discovered by Villiers as early as 1891,⁴⁰ and ever since CDs have undergone extensive studies in a variety of fields, such as analytical chemistry,⁴¹ enzyme technology,⁴² catalytic reactions,⁴³ and pharmaceuticals.^{44,45} The easy availability of CDs from their starch precursors, like potato, rice, and corn, endowed CDs with inexpensive cost for vast applications. Within the CD family, the ones containing 6, 7, and 8 glucose units are most commonly used, named as α -, β -, and γ -CD, respectively. The CDs have a truncated cone-resembled shape with a hollow cavity. The sizes of the primary and secondary sides of the CDs are dependent on the unit number of glucose (Table 1). The depth of the hollow cavity is 0.78 nm for all three types of CDs. The hydroxyl groups of the glucose units are oriented toward the outside at the orifice of the two ends, while methinic protons are located inside the cavity, the structure of which enables CDs with hydrophilic external surface and hydrophobic hollow cavity. Thus, a variety of guests can be encapsulated into the cavity via the host–guest interaction in aqueous conditions and even in the solid state, which include

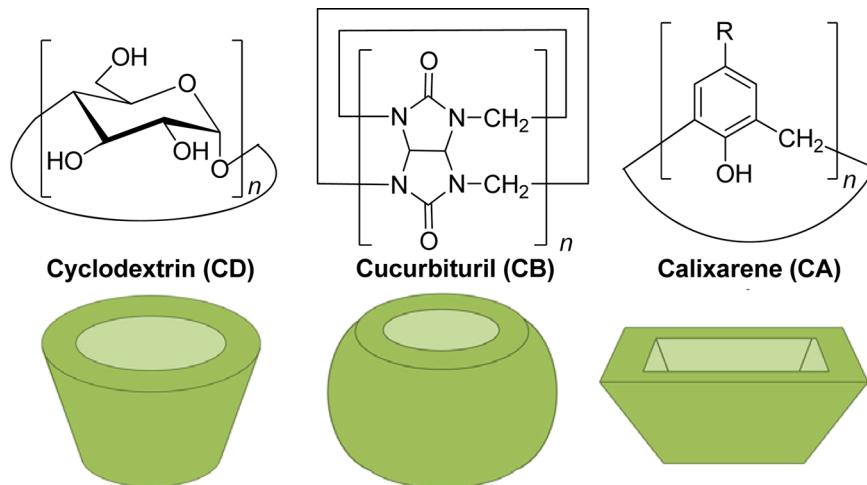


Figure 1. Chemical structures and schematic representations of cyclodextrin (CD), cucurbituril (CB), and calixarene (CA).

Table 1. Dimensions of Cyclodextrins (CDs), Cucurbit[*n*]uril (CB[*n*]), and Calix[*n*]arene (CA[*n*])^{33,56–58}

macrocyclic molecules	internal diameter (nm)	external diameter (nm)	height (nm)
α-CD	0.57	1.37	0.78
β-CD	0.78	1.53	0.78
γ-CD	0.95	1.69	0.78
CA[4]	0.30	0.590	1.175
CA[6]	0.76	0.496	1.624
CA[8]	1.17	0.923	2.24
CB[5]	0.44	0.24	0.91
CB[6]	0.58	0.39	0.91
CB[7]	0.73	0.54	0.91
CB[8]	0.88	0.69	0.91

small molecules,⁴⁶ cationic or anionic guests,⁴⁷ proteins,^{48,49} and polymer chains.^{50–53} The formation and dissociation of the host–guest complexes are closely related to the kinetic and thermodynamic properties of the complexes, the sizes of the host and guest, as well as the environmental conditions (pH and temperature), which provide the possibilities for the responsiveness of the host–guest systems.^{46,47,54,55}

Because of the natural availability from starch, CDs exhibit good water solubility, good biocompatibility, and nontoxicity toward biological systems.⁵⁹ These beneficial properties have further fostered extensive studies on using CDs in biomedical field. In the early stage, researchers mainly focused on utilizing bare CDs or simple CD derivatives. For instance, after obtaining the evidence of using CDs as enzyme models by several pioneer researchers, like Bender,⁶⁰ Breslow,⁶¹ Tabushi,⁶² and Saenger,⁶³ much research effort has been made to introduce catalytic groups onto CDs to build artificial enzymes for biomimetic reactions.⁶⁴ By forming the host–guest complexes between drugs and CDs or CD derivatives, the water solubility of hydrophobic drugs can be greatly increased, thereby enhancing the drug availability in biological systems. This is the direct application of CDs for drug delivery.⁶⁵ For better pharmaceutical uses, chemical modifications on CDs were carried out to further improve their solubility, drug encapsulation ability, and drug release capability, while minimizing the toxicity of the CDs. In this aspect, a variety of functional groups were directly modified onto CDs. Cationic CDs were synthesized by introducing amino containing groups onto the primary side of the CDs.^{66,67} Anionic CDs were

produced by attaching carboxymethyl groups or sulfonic groups onto the CDs.^{68–70}

On account of the superiority of nanosystems in biomedical field, the construction of CD-based supramolecular assemblies or aggregates in nanoscale size has drawn significant attention. For example, such kind of supramolecular systems can be developed into various morphologies under different conditions due to their versatile and reversible building blocks (Figure 2), which is an obvious advantage over traditional materials. Thus, the supramolecular systems have shown wide applications in biomedical applications such as drug/gene delivery and bioimaging.^{71–74} Recently, several excellent review articles summarized the formation and related applications of CD-based supramolecular assemblies. Jiang and coauthors discussed the construction strategy of supramolecular self-assemblies formed by CD-based complexation, which include the micelle formation by CD modified polymers, layer-by-layer hollow microcapsules, reversible micelles and vesicles, as well as the polymer hydrogels formed by the host–guest inclusion.⁷⁵ Another review covered the topic of CD-based bioactive supramolecular assemblies and their related biological applications, including the fluorescence sensing, solubilization of drug molecules for delivery, biomolecular interactions, as well as assemblies mediated by nanoparticles, polymers, or carbon nanotubes.⁷⁶ More specifically, Benito and Tetsumi gave a relatively comprehensive discussion on CD-based gene delivery systems. Taking advantage of good biocompatibility, ease chemical modification, and facile host–guest complex formation of CDs, a variety of CD-based gene carriers were developed.^{77,78} Very recently, Ma et al. discussed the drug delivery by CD-based supramolecular systems.⁷⁹ Pharmaceutical and biomedical applications of CD-based supramolecular hydrogels were also highlighted.^{80,81} Therefore, a lot of research has demonstrated the significant roles of CDs in supramolecular chemistry, especially for the preparation of CD-based supramolecular assemblies for biological applications.

1.2.2. Calix[*n*]arenes (CAs). In the family of macrocyclic molecules, CAs have established their own status as the third generation of macrocyclic molecules. They are also listed as one of the three major macrocyclic molecules together with CDs and crown ethers. Typically, CAs are produced by chemical synthesis between phenols and formaldehyde with phenolic units linked by methylene bridges at the meta-positions, which are different from

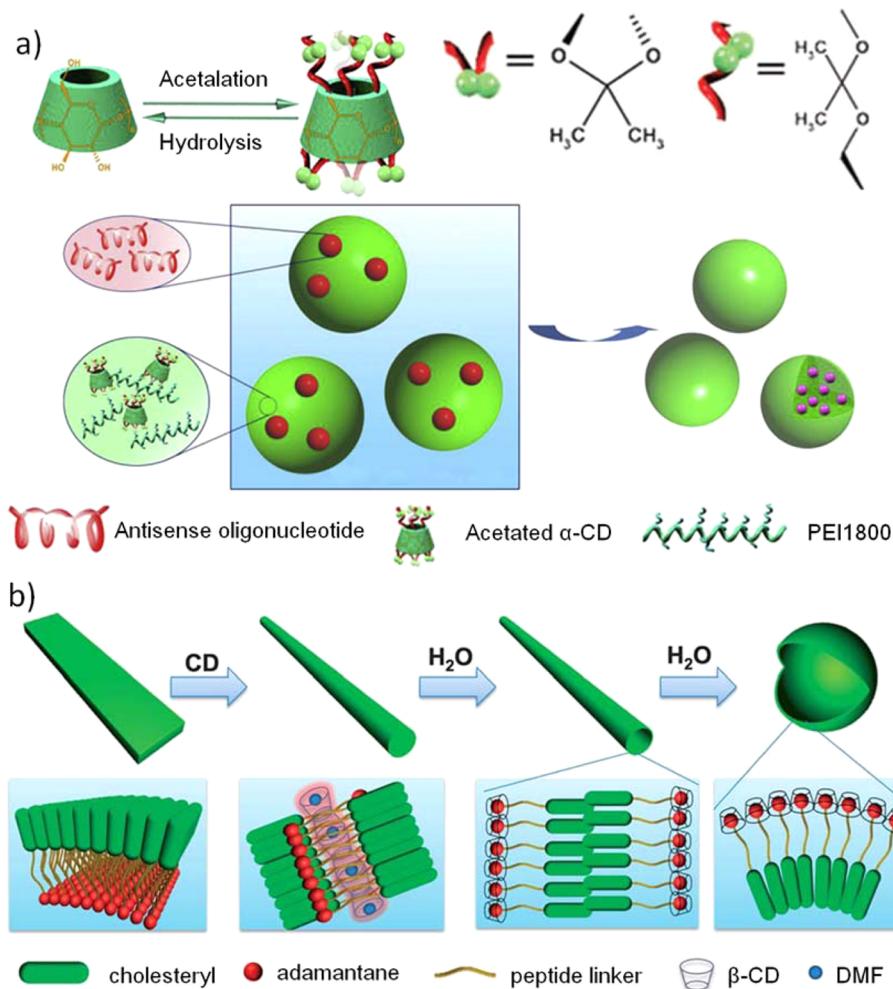


Figure 2. Supramolecular material synthesis and nanoparticle constructions based on CDs. (a) Synthesis of acetylated- α -CD by kinetically controlled acetalation and fabrication of hybrid nanoparticles. Reprinted with permission from ref 71. Copyright 2013 Elsevier. (b) Supramolecular morphology evolution of β -CD-based nanohybrids. Reprinted with permission from ref 72. Copyright 2013 The Royal Society of Chemistry.

the naturally available source of CDs. Because CAs with even numbers ($n = 4, 6$, and 8) can be synthesized by one-step reaction and easily purified, they have been widely investigated. On the other hand, CAs with odd numbers ($n = 5, 7$, and 9) are relatively difficult to synthesize, resulting in less usage of these CAs. CAs also possess a corn-resembled shape with a hollow cavity, as well as two rims at the primary and secondary sides. The distinguished character is that CAs have variable cavity dimensions according to the number of incorporated phenolic units.^{82,83} The lower rim of CAs is featured with phenolic oxygen and thus has a hydrophilic property, while the upper rim is hydrophobic due to the presence of methyl groups. The guests can be incorporated into the cavity at both rims of CAs, which include small organic molecules, ions, sugars, proteins, and so on.⁸⁴ Such inclusion complexation is driven by various forces such as hydrophobic effect, ion-dipole interaction, and hydrogen-bonding interaction.

As compared to CDs, less study has been reported in terms of biomedical applications of CAs,⁸⁵ which is mainly due to hydrophobic property and poor water solubility of bare CAs. Even in nonpolar organic solvents where CAs are well dissolved, strong competition between organic solvents and guest molecules can still hamper the host–guest complex formation.⁸⁶ To address this problem, researchers have spent considerable effort to prepare hydrophilic and water-soluble CAs. Sulfonation

at the rims of CAs was a common strategy to prepare water-soluble CAs.⁸⁷ In addition, the conjugation of carboxylic acid groups onto the lower rim⁸⁸ and the functionalization of polar groups onto the edge of CAs were also carried out for the same purpose.^{89,90} By using click chemistry, cationic, anionic, and nonionic CA derivatives with a good water solubility were efficiently synthesized.⁹¹

Through the functionalization process, macrocyclic molecules can be imposed with multifunctional capabilities while maintaining their property of encapsulating guest molecules. For instance, poly(pyridinium) salts were functionalized onto CAs, aiming at biosensing application (Figure 3a).⁹² Both the lower and the upper rims of CAs could be functionalized with water-soluble groups, including the conjugation with amino containing cationic units for the DNA condensation and gene delivery (Figure 3b).^{93,95–97} 4-Butylsulfonato pendant groups were also modified onto CAs to achieve water-soluble CAs and constructed micelles by a new amphiphile (Figure 3c).⁹⁴

In terms of biocompatibility of CAs, water-soluble CAs derivatives, like *para*-sulfonato-calix[4]arene, exhibit low toxicity, and the *in vivo* dosage can reach up to 100 mg kg^{-1} without a toxic effect in mice.⁹⁸ Thus, CA derivatives stand out as an alternative for CDs, being the host molecules to encapsulate a variety of guest molecules for biomedical applications, including

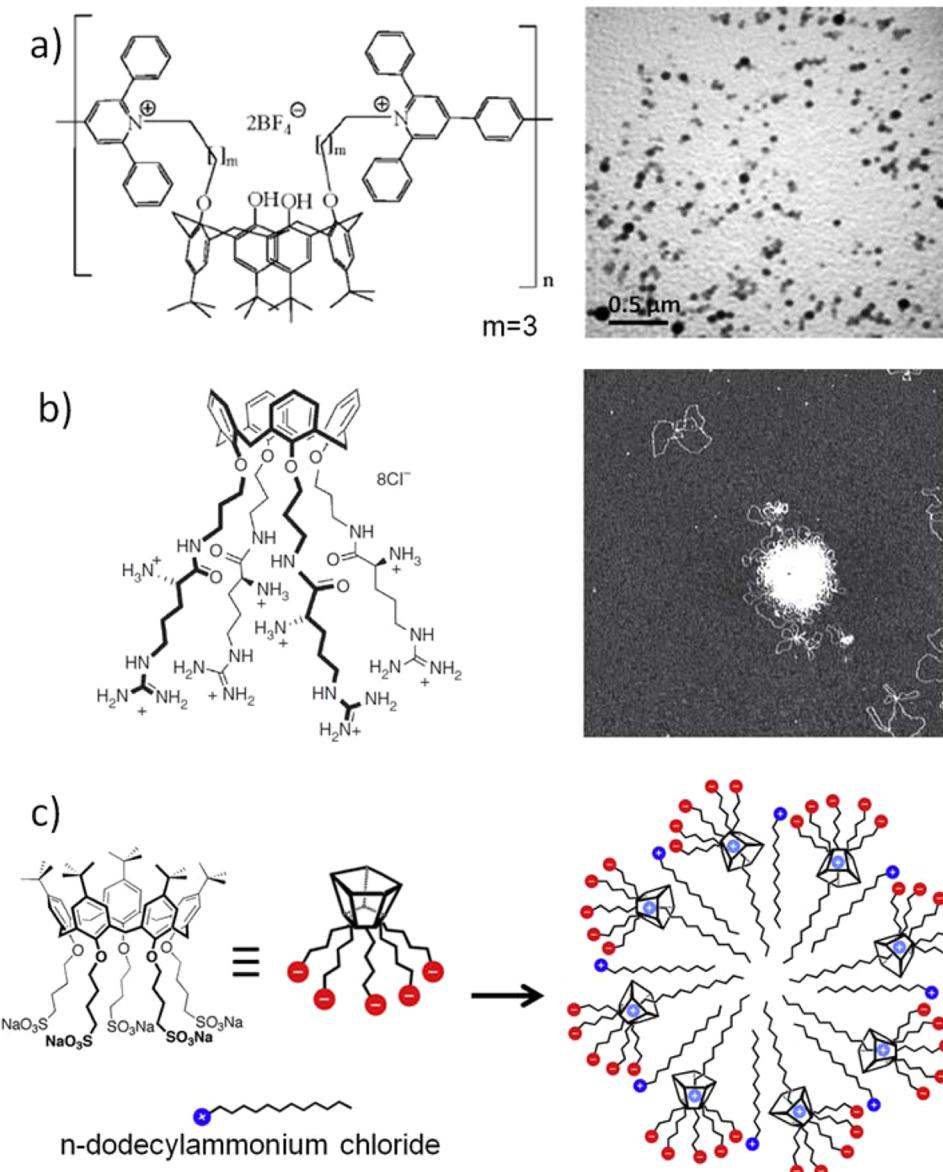


Figure 3. (a) Chemical structure of poly(pyridinium) salt containing calix[4]arene unit (left) and its interaction with DNA studied by TEM (right). Reprinted with permission from ref 92. Copyright 2009 The Royal Society of Chemistry. (b) pEGFP-C1 plasmid DNA folding by incubation with 1 mM argininocalixarene derivatives (left) for gene delivery. AFM image represents a $2 \times 2 \mu\text{m}$ scan (right). Reprinted with permission from ref 93. Copyright 2013 Nature Publishing Group. (c) A supramolecular micelle formed from water-soluble calix[5]arene bearing 4-butylsulfonato pendant and n -dodecylammonium chloride. Reprinted with permission from ref 94. Copyright 2013 Elsevier.

the solubilization of hydrophobic drugs, biomimics of ion channels, biochemical recognition, drug/gene delivery, and enzymatic activity.^{56,84} In addition to the host–guest complexation, CAs and their derivatives have shown interesting inhibition activities toward virus,⁹⁹ bacterial,^{100,101} fungus,¹⁰² and some cancers.¹⁰³ A review article by Fatima discussed the usage and biological activities of CAs as a new type of chemical reagents.¹⁰⁴ Kim summarized the chemical functionalization strategies of CAs, as well as their related biological applications, such as on-chip DNA detection, drug/gene delivery, and drug discovery.⁸⁴

Upon the development of CA derivatives with good water solubility, the formation of supramolecular structures by encapsulating guest molecules into CA cavity has become an interesting topic. The binding constants of CAs with guest molecules are usually higher than that of CDs, and Nau and coauthors discussed the availability of guest drugs encapsulated

into the cavities of CAs.¹⁰⁵ Moreover, variable functional groups on the upper or lower rims can endow CA derivatives with special recognition capabilities toward ionic species. Recently developed strategies for the formation of CA-based supramolecular systems were described in a review article by Liu and coauthors.¹⁰⁶ On the basis of the strong host–guest inclusion and strong recognition capability, supramolecular assemblies such as capsules were prepared for biomedical applications, which will be addressed in detail in a following section. Nevertheless, biomedical application of CA-based supramolecular systems has become a rising research topic during recent years, showing promising application potential in this area.

1.2.3. Cucurbit[*n*]urils (CBs). In comparison with CDs and CAs, the family of CBs has a relatively short history in supramolecular chemistry. Although CBs were first discovered by Behrend and co-workers as early as 1905 when they

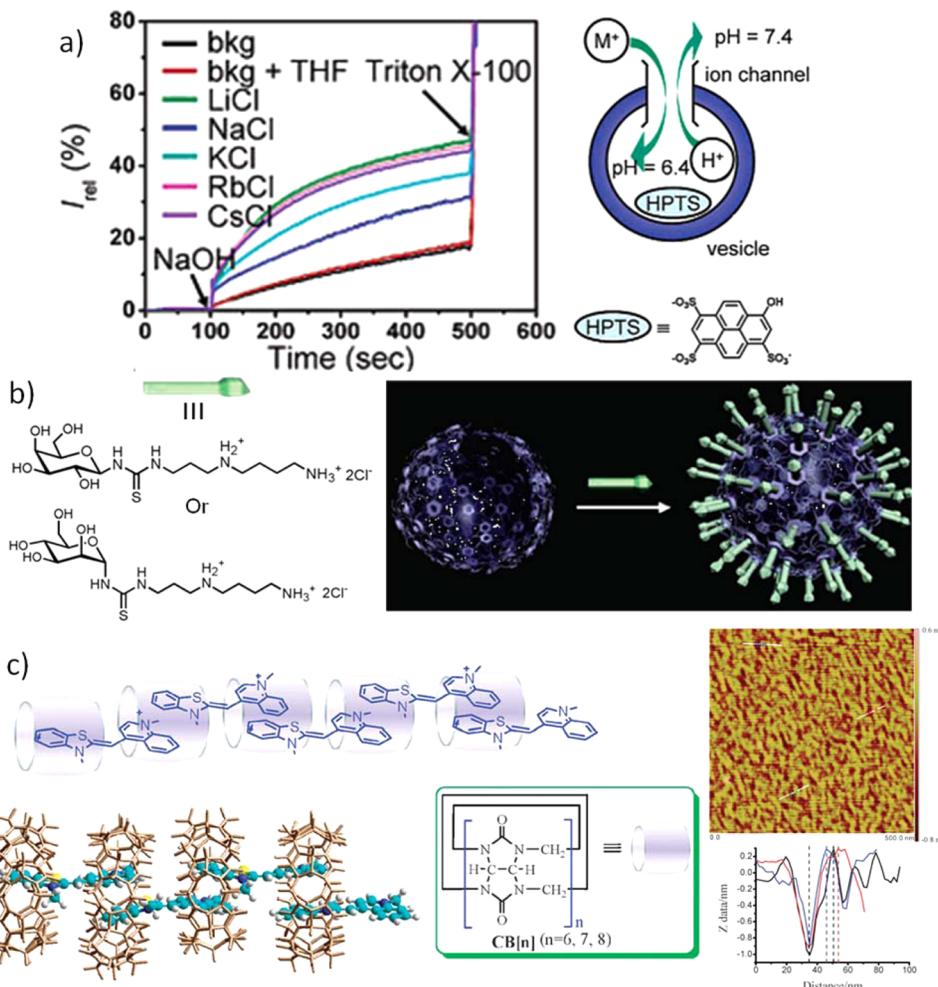


Figure 4. (a) Changes in the fluorescence intensity ratio (I_{460}/I_{403}) as a function of time associated with alkali metal ion transport across the CB-based vesicle membrane. Reprinted with permission from ref 124. Copyright 2004 American Chemical Society. (b) Representative illustration of the facile surface modification of the vesicle through host–guest chemistry between CB and polyamine tags. Reprinted with permission from ref 125. Copyright 2005 American Chemical Society. (c) Schematic illustration of the TO (thiazole orange)-CB[8] assembly (left), and AFM image and cross-section profile of TO-CB[8] spin-coated on the mica surface. Reprinted with permission from ref 126. Copyright 2011 The Royal Society of Chemistry.

conducted the condensation of glycoluril and formaldehyde under acidic conditions, the chemical structure of the white compound they obtained remained unrevealed.¹⁰⁷ The answer to the chemical nature of CBs was finally given in 1981 when Mock and co-workers repeated Behrend's work,¹⁰⁸ by forming the complexation with calcium sulfate. As one important member of macrocyclic compounds, CBs consist of glycoluril units connected by methylene groups. Although its synthetic route by acidic condensation was discovered one century ago, the isolation of major CB members was achieved only in recent decades. Initially, the existence of other CB members (i.e., CB[5], CB[7], and CB[8]) with CB[6] was known, but the isolation and identification were accomplished by several research groups around the year of 2000. Kim and co-workers successfully isolated CB[5], CB[7], and CB[8].¹⁰⁹ Day and co-workers isolated CB[5] that was entrapped in the cavity of CB[10].^{110,111} Next, Issacs and co-workers obtained CB[10] by replacing CB[5] with melamine diamine.¹¹² Despite the relatively short history, CBs have undergone a quick development in supramolecular chemistry, indicated by the increasing amount of publications in the past decades.^{58,113,114}

CBs have a pumpkin-resembled shape with two hydrophilic carbonylated rims and a hydrophobic cavity that is capable of

hosting guest molecules. The cavity depth of CBs is 0.91 nm (Table 1). The cavity sizes of CB[6], CB[7], and CB[8] are similar to those of α -, β -, and γ -CD, respectively. The width of CBs is varied, dependent on the number of glycoluril units. For instance, the portal diameter of CB[10] ranges from 0.9 to 1.1 nm, and its internal diameter of the cavity is from 1.07 to 1.26 nm. The cavity volume of CB[10] is almost twice that of CB[8].¹¹² The cavity of CBs is nonpolar and hydrophobic due to the absence of bonds or lone pairs inside the cavity. Thus, the internal hydrophobic cavity of CBs is capable of encapsulating neutral molecules. Positively charged species can also be encapsulated via ion-dipole interactions with carbonylated portals. CBs can provide the hydrogen-bonding interactions between protonated molecules and carbonyl portals.¹¹⁵ The variable size of the portal and cavity in different CB members leads to different recognition properties. For instance, CB[6] is known to be able to host alkyl ammonium ions, while CB[7] can host larger molecules that are too big to be encapsulated by CB[6], such as 2,6-bis(4,5-dihydro-1H-imidazol-2-yl)naphthalene and adamantanamine. CB[8] is even able to host two molecules by forming a 1:2 host–guest complex, such as two 2,6-bis(4,5-dihydro-1H-imidazol-2-yl)naphthalene molecules.¹¹³ Almost all of the CB members are insoluble in organic solvents. By forming

the host–guest complexes with positively charged organic guests, such as *p*-xylylene diammonium, some CB-based host–guest complexes become readily dissolved in organic solvents or water.¹¹⁶ In supramolecular chemistry, CBs are known for their extremely high affinity toward some guest molecules. An affinity as high as 10^{12} M^{-1} was reported by Isaacs et al. in 2005,¹¹⁷ and, afterward, even higher affinity reaching 10^{15} M^{-1} was reported.^{118,119}

With regard to the biomedical applications, water solubility of CBs is more concerned. Their water solubility is one bottleneck, limiting their applications in biomedical field. Only moderate water solubility is observed for CBs, which is similar to that of β -CD. The water solubility of CBs can be partially enhanced in acidic conditions or in the presence of alkali metal ions. To further overcome their poor water solubility, researchers have made great efforts to introduce functional groups onto CBs, which was not an easy task in the CB chemistry. There are a few established strategies for the CB functionalization, including direct condensation with glycoluril derivatives or aldehyde derivatives bearing functional groups during the CB formation,^{120–122} and postgrafting of functional groups onto as-prepared CBs.¹²³ Kim and co-workers summarized the functionalization routes for CBs in an early review published in 2007.⁵⁸ They also discussed related applications of functionalized CBs, such as CB-based ion channels (Figure 4a),¹²⁴ supramolecular vesicles (Figure 4b),¹²⁵ supramolecular polymers (Figure 4c),^{126–128} and chromatography.¹²⁹ Because these practical functions were realized by supramolecular host–guest interactions in a facile way, the superiorities of supramolecular systems were clearly demonstrated.

Although comprehensive toxicity studies for CBs are still under investigation, previous research work indicated good safety of using CBs in biological systems. In vitro cell study showed that no cytotoxicity was observed at high dosage up to millimolar ($\text{IC}_{50} = 0.53 \text{ mM}$) using Chinese hamster ovary CHO-K1 cells.³⁷ No harmful effect was found for the intracellular presence of CBs, and in vivo study in mice demonstrated that the dosage of CB[7] up to 250 mg kg^{-1} showed little toxicity effect.³⁷ CBs exhibited nontoxic effect at a high concentration (1.0 mM) toward human kidney HEK293, human hepatocyte HepG2, and murine macrophage RAW264.7 cell lines.³⁸ By using fluorescence labeled CB[7] and CB[8], intracellular uptake crossing cell membrane was observed.¹³⁰ Although using CBs in biomedical field has only started few years ago, such research has attracted a lot of attention in supramolecular chemistry. By forming simple host–guest complexes between CBs and drug molecules, the solubility of the drugs^{131,132} or CBs themselves¹³³ can be significantly increased, and thus the bioavailability of the drugs in biological environment can be improved. In some cases, however, strong host–guest complexation by CBs prevented guest molecules from release into the surrounding environment, which might bring new problems for the drug delivery application of such host–guest systems.¹³⁴ By employing the host–guest interaction strategy, supramolecular vehicles or nanoparticles based on CBs showed several advantages in biomedical applications, such as ease of fabrication, targeted drug/gene delivery, and responsive release, which will be discussed in detail later.

In addition to CDs, CAs, and CBs, another type of macrocyclic molecule, pillar[n]arene, has an even shorter history in supramolecular chemistry, and it was first reported by Ogoshi and co-workers in 2008.¹³⁵ Since then, pillar[n]arene has been investigated as a novel host in supramolecular chemistry and

employed in various applications.^{136–139} Because of its special symmetric structure and easy functionalizations,^{140–142} pillar-[n]arene has been utilized to fabricate various supramolecular systems.^{143–145} In this Review, biomedical applications of this new type of macrocyclic molecule will also be discussed in the following sections.

2. BIOMEDICAL APPLICATIONS

This Review mainly highlights the current research progress on biomedical applications of host–guest interaction-based supramolecular systems. Three major types of macrocyclic molecules, that is, CDs, CAs, and CBs, are the discussion focus. Other types of macrocyclic molecules, like pillar[n]arene, are also discussed in certain sections. The specific biomedical applications include drug delivery, gene delivery, drug/gene codelivery, bioimaging, as well as photodynamic therapy.

2.1. Drug Delivery

Although lots of anticancer drugs have been developed during the past century, effective cancer treatment is still facing considerable difficulties due to at least the lack of efficient drug delivery strategies. For example, hydrophobic drugs with a low solubility in aqueous solution severely limit their applications in biological environment. In addition, the side effects of non-targeted drug delivery might cause unwanted damage to healthy or normal tissues instead of focusing on cancerous tumor sites. The cancer cell membrane naturally sets up the last barrier for anticancer drug delivery into cancer cells. Therefore, drug delivery systems with new functions are needed for the enhancement of anticancer drug solubility in aqueous environment, targeted drug delivery into tumor sites, and effective internalization into cancer cells. Supramolecular chemistry strategies have been utilized to achieve these targets in drug delivery.¹⁴⁶

2.1.1. Direct Host–Guest Systems between Macroyclic Molecules and Drugs. In fact, more than one-half of newly developed chemical drugs have low water solubility, although they show great potentials in cancer therapy.^{147,148} Thus, it is urgently required to enhance the solubility or dissolution of drugs in aqueous conditions. In the scope of supramolecular chemistry, a directive method is using the host–guest complexation between water-soluble macrocyclic molecules and drugs to enhance the water solubility of drugs. In addition, by forming such host–guest complexes, it is capable of protecting drug molecules from chemical reactions and photochemical/thermal degradation in biological environment. Chemical activity of delivered drugs might be altered by the host–guest inclusion as well. On account of the host–guest inclusion, the encapsulated drugs can be released sustainably from the cavity of macrocyclic molecules, achieving prolonged therapeutic effect. Upon external stimulus, such as thermal change, pH variation, or competitive binding, to disassociate the host–guest complexes, delivered drugs can be released in a controlled manner.¹⁴⁹ In a review article, Nau et al. discussed the enhancement of bioavailability of drugs by the host–guest complexation with macrocyclic molecules.¹⁰⁵ Up to now, such host–guest complexation has been successfully demonstrated as a general approach to enhance the water solubility of anticancer drugs, aiming at better chemotherapeutic efficacy.

2.1.1.1. Drug–CD Complex. In early studies, CDs and their derivatives were directly used as the drug hosts in drug delivery.^{35,36,65,77,150} Uekama and coauthors discussed improving drug solubility and stability by direct complexation with CDs,

and highlighted various delivery routes.⁷⁷ The interactions between CDs and drug molecules with different charges were investigated by Okimoto and coauthors.¹⁵¹ At present, over 30 kinds of commercialized pharmaceutical products by CD-based complexes could be found on the market.⁴⁵ During recent years, only a few studies about the applications of simple host–guest complexes between CDs and drugs in biomedical field were reported. For instance, β -CD and its derivative, 2-hydroxypropyl- β -CD, were used to improve the water solubility of delta-8-tetrahydrocannabinol to enhance its ocular bioavailability.¹⁵² On the other hand, many new types of CD-based nanomaterials were reported by either using supramolecular self-assembly to form nanosized aggregates or combining with other functional materials to afford novel nanohybrids, which paved new ways for the exploration of CDs in biomedical applications. Among these supramolecular nanosystems, the host–guest inclusion between CDs and drug molecules is still essential for the drug delivery. For example, anticancer drug paclitaxel (PTX) was encapsulated into β -CD. The host–guest complex (β -CD:PTX = 1:1) was further self-assembled into biodegradable capsules, which were used to treat breast cancer cells (Figure 5a).¹⁵³ In another report, the host–guest complex (β -CD:PTX = 1:1) was directly used to construct a vesicle for drug delivery (Figure 5b).¹⁵⁴

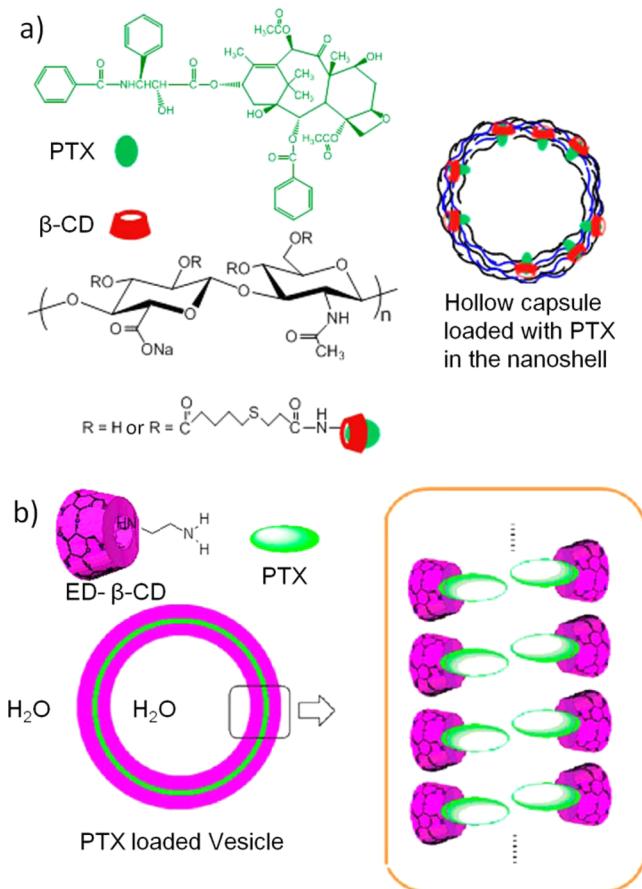


Figure 5. (a) Directly using the β -CD/PTX complex to form biodegradable capsules. Reprinted with permission from ref 153. Copyright 2013 American Chemical Society. (b) Vesicle formation by direct host–guest complexation between PTX and mono[6-(2-aminoethyleneamino)-6-deoxy]- β -CD (ED- β -CD). Reprinted with permission from ref 154. Copyright 2012 American Chemical Society.

2.1.1.2. Drug–CB Complex. Because of good biocompatibility and strong affinities toward some guest molecules, CBs have also been utilized to host a variety of drugs for the purpose of improved drug delivery. Physical stability of pyrazinamide (pyrazine-2-carboxamide) and isoniazid (isonicotinohydrazide) was reported to be improved by the inclusion into the cavity of CB[7] for the treatment of tuberculosis (Figure 6a).¹⁵⁵ Antitumor activity of CB[8]–fullerene complex was investigated.¹⁵⁶ The chemical structure of the inclusion complex between CB[7] and triamterene, that is, 2,4,7-triamino-6-phenylpteridine, was investigated by Ma and co-workers. Pharmacokinetics study revealed that the host–guest complex could prolong the $t_{1/2}$ value from 1.42 to 2.62 h post oral administration (Figure 6b).¹⁵⁷ Collins et al. used CB[7] to encapsulate anticancer drug dinuclear platinum complex and studied its reaction rate, cytotoxicity, and interaction with DNA.^{153,158,159} They also used CB[6], CB[7], and CB[8] to complex albendazole and increased its aqueous solubility by 2000-fold.¹³¹ Enhanced cytotoxicity of albendazole derivatives by forming the host–guest complexes with CB[7] and CB[8] was also reported.¹³² Huang and co-workers used CB[7] and CB[8] to bind gefitinib, an inhibitor toward epidermal growth factor receptor(EGFR) for lung cancer treatment. Gefitinib has poor solubility in neutral pH and thus poor oral absorption, which was improved by the host–guest complexation with CB[7] or CB[8]. The study revealed an enthalpy controlled complex formation process, implying that the inclusion was driven by hydrophobic and van der Waals interactions (Figure 6c).¹⁶⁰

Platinum-based complexes are a class of widely used anticancer drugs, but their application was limited by poor water solubility. Wheate and co-workers employed CBs to improve the water solubility of platinum complexes for the drug delivery against cancer cells.^{161,162} To address the systematic toxicity and drug resistance of a platinum-based drug, cisplatin, in the cancer treatment, they encapsulated cisplatin by CB[7] and achieved enhanced cytotoxicity in human ovarian carcinoma cell lines. In vivo study revealed the CB–cisplatin complex can be used for the treatment of drug resistance human cancer, and the pharmacokinetic effect of CB[7]–cisplatin complex (Figure 7a,b) was indicated to be responsible for overcoming the cisplatin drug resistance. The in vivo treatment showed a promising application on account of the possible decrease of initial drug exposure.¹⁶³ By using density functional theory, Venkataraman and co-workers investigated the host–guest interaction between CBs and platinum-based anticancer drugs including oxaliplatin, nedaplatin, carboplatin, and cisplatin from a theoretical point of view.¹⁶⁴ From the calculated enthalpy and Gibbs free energy during the complex formation, CB[7]–drug complexation process was proven to be spontaneous and energy-favorable. They also exhibited the charge transfer within the inclusion complex from the high chemical shielding in the computed 1 NMR spectra. Hydrogen-bonding interactions between amine protons in the platinum-based drugs and oxygen portal in CBs contributed to the complexation. In addition to the enhancement of water solubility, Appel and co-workers encapsulated temozolomide (TMZ), a primary chemotherapeutic drug against glioblastoma multiforme (GBM), into the hydrophobic cavity of CB[7], which could decrease the degradation rate of TMZ and thus prolong its lifetime in physiological environment. Moreover, the CB[7]–TMZ complex could effectively bypass the blood–brain barrier with improved anticancer efficacy toward GBM cell line (Figure 2c).¹⁶⁵

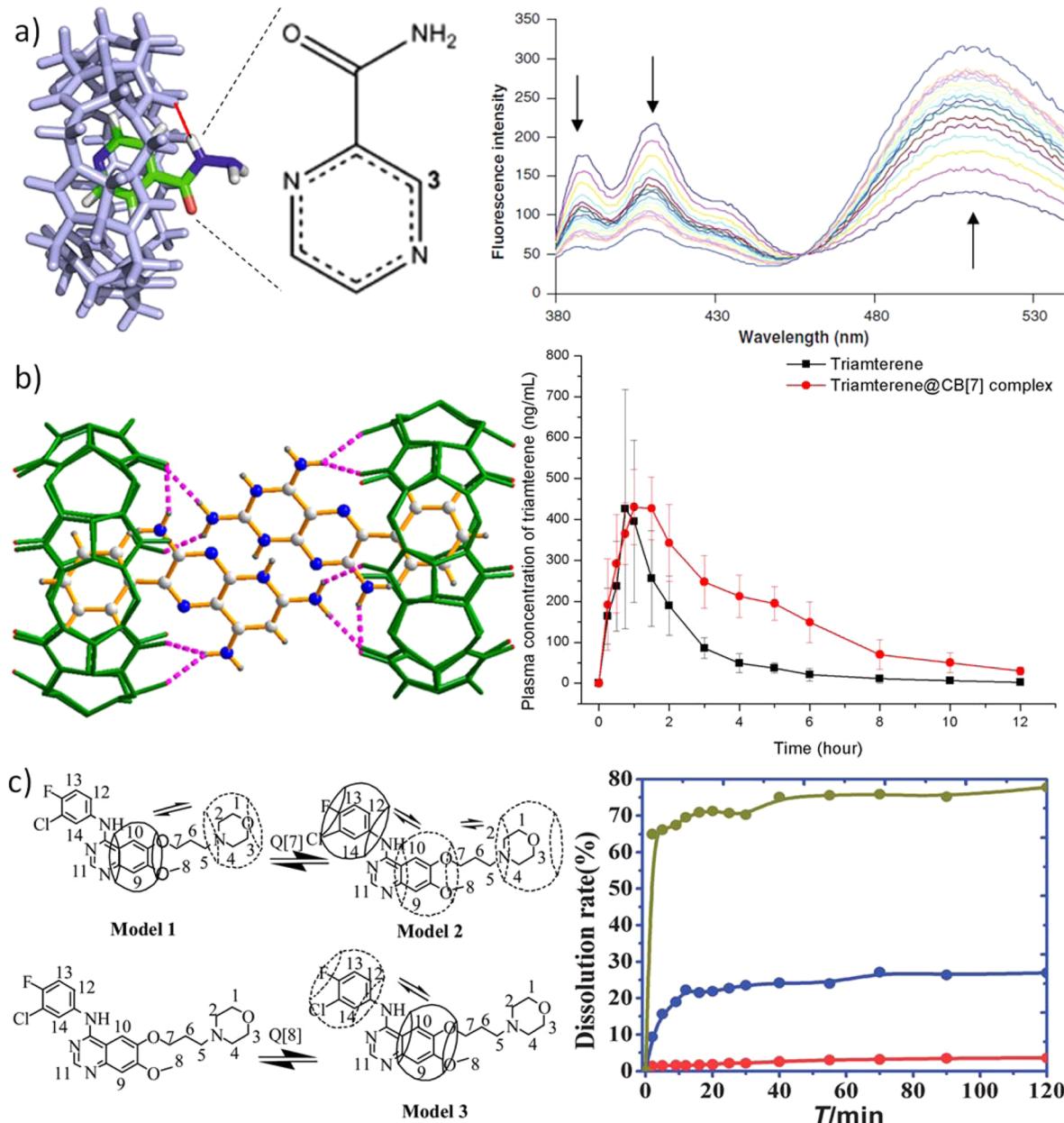


Figure 6. (a) Host–guest structure of isoniazid with CB[7] (left). Intermolecular hydrogen bond is shown as a red line. The fluorescence spectra ($\lambda_{\text{Ex}} = 374 \text{ nm}$) of 2-aminoanthracene (5 μM) at pH 1.5 in the presence of 1 mol equivalent of CB[7] upon increasing the isoniazid concentration (right). The arrows indicate the direction of changes. Reprinted with permission from ref 155. Copyright 2010 Springer. (b) A capsule-shaped dimer between CB[7] and triamterene (left), and mean plasma concentrations versus time profiles of triamterene by following oral administration of free triamterene and triamterene@CB[7] complex to male rats (right). Each point represents the mean \pm SD ($n = 5$). Reprinted with permission from ref 157. Copyright 2013 American Chemical Society. (c) Possible interaction modes between CB[7,8] and gefitinib (left), and the dissolution rate curves (right) of gefitinib (red), a physical mixture of CB[7] and gefitinib (blue), and their inclusion complex (green). Reprinted with permission from ref 160. Copyright 2014 The Royal Society of Chemistry.

Very recently, Isaacs and co-workers developed a new class of drug containers based on acyclic CBs. Through direct host–guest complexation with drugs, the prepared container was capable of significantly increasing the solubility of 10 water-insoluble drugs by various extents, that is, up to 2750 for certain drug molecules (Figure 8).^{166,167} They also synthesized a series of CB derivatives bearing different charges by introducing varied solubilizing groups (SO_3^- , OH , NH_3^+), and studied their complexation activity in solubilizing cationic, anionic, and neutral drugs.¹⁶⁸ Moreover, this group prepared biotin-functionalized CB[7], which was proven to be able to host a variety of

anticancer drugs, including albendazole, tamoxifen, camptothecin (CPT), irinotecan, and temezolomide.¹⁶⁹ The targeting ligand, biotin, endowed the delivery system with selective capability to cancer cells with overexpression of biotin receptors, which was supported by the *in vitro* study with L1210FR cells.

2.1.1.3. Drug–CA Complex. Although CAs are inherently hydrophobic and not suitable for enhancing the water solubility of hydrophobic drugs through direct complexation,⁸⁶ many attempts have been made to produce hydrophilic CA derivatives for such application.

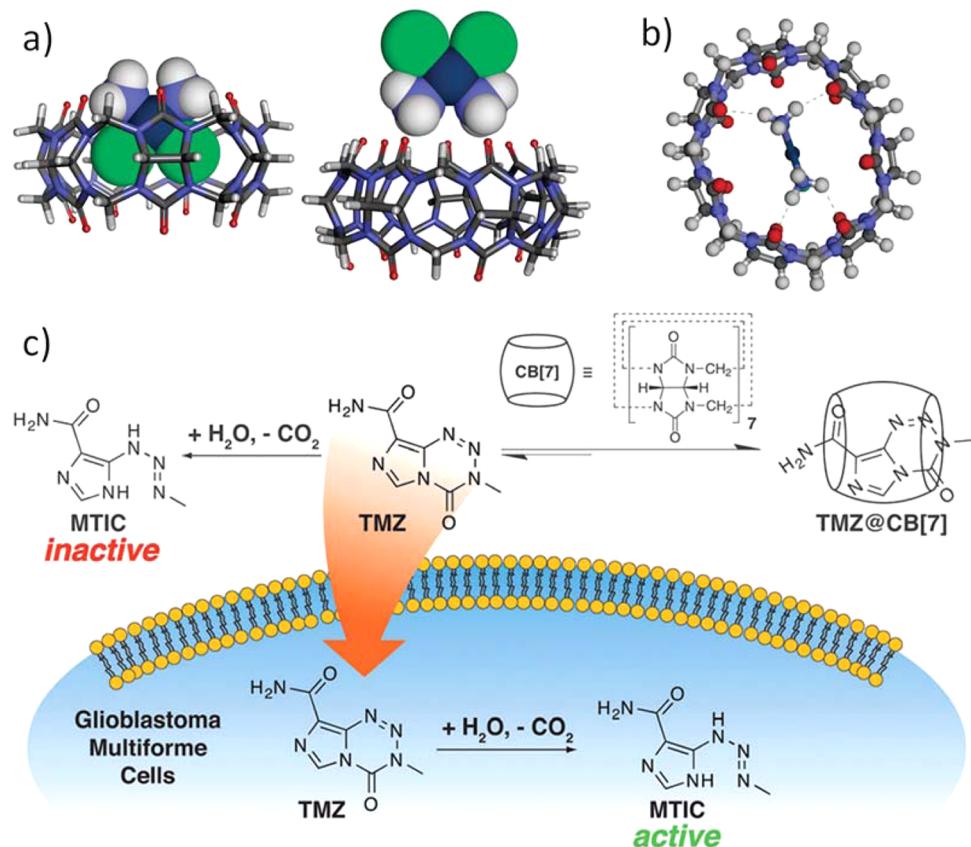


Figure 7. (a) Molecular models of the host–guest complexes between anticancer drug cisplatin and CB[7], showing two potential binding modes: pointing-in, where the platinum atom and chlorido ligands are located within the macrocycle cavity, and pointing-out, where the binding occurs only at the CB[7] portals and is less energetically favorable. (b) A molecular model of the pointing-in mode of the cisplatin binding into CB[7], showing that four hydrogen-bonding interactions from the amine hydrogen atoms of the drug to the carbonyl oxygen atoms of CB[7] (bond lengths: 2.15, 2.22, 2.38, and 2.44 Å) stabilize the host–guest complex. Reprinted with permission from ref 163. Copyright 2012 Royal Society of Chemistry. (c) Degradation of temozolomide (TMZ) to 5-(3-methyl-triazen-1-yl)imidazole-4-carboxamide (MTIC) outside of the cell, and the stabilization mechanism of TMZ through the complexation with CB[7]. Reprinted with permission from ref 165. Copyright 2012 Royal Society of Chemistry.

Sulfonation at the rim of CAs has been widely utilized to produce water-soluble CA derivatives, which served as important containers to encapsulate drugs with poor water solubility in drug delivery. The chemical structures of various drugs that were encapsulated into *para*-sulphonatocalix[4]resorcinarene (PSC[4]R) were illustrated in Figure 9. For instance, Menon and co-workers prepared PSC[4]R to encapsulate poorly soluble drug mycophenolate mofetil (MMF) by forming the host–guest complex (PSC[4]R:MMF = 2:1).¹⁷⁰ A similar strategy was used to solubilize another water insoluble drug, carvedilol (CDL),¹⁷¹ as well as to enhance the water solubility of a topoisomerase I inhibitor topotecan (TPT) by 5-fold.¹⁷² Recently, they used PSC[4]R to improve the water solubility and bioactivity of lamotrigine (LMN). In comparison with free drug, the formed PSC[4]R–drug complex improved the in vitro dissolution of the drug, while the in vivo acute oral toxicity was decreased.¹⁷³ Meanwhile, Yilmaz and co-workers fabricated *O*-phosphorylated calix[n]arenes and *P*-phosphorylated CAs to host and solubilize some neutral molecules, such as nifedipine, niclosamide, and furosemide, via the host–guest interaction.^{174,175} Their study indicated that the solubility of the above-mentioned guest molecules could be enhanced significantly by the CA derivatives as the host molecules. The results also indicated that many factors, including hydrophobic cavity diameter, hydrogen binding ability, and cavity rigidity, might influence the drug-solubilizing capability of the prepared *O*-phosphorylated CAs.

Furthermore, circular dichroism was used to study the inclusion mechanism between achiral CAs and chiral pharmaceutical reagents in different solvents, such as acetonitrile, methanol, and water. The obtained results suggested that CAs could act as ideal hosts to encapsulate various unit by both upper and lower rims.¹⁷⁶ By employing the spectrometry and ¹H NMR techniques, it was found that the inclusion between *p*-sulfonatocalix[6]arene and vitamin B6 was mainly driven by hydrophobic and hydrogen-bonding interactions assisted by electrostatic interaction. Such research provides useful information for future design of macrocyclic molecules in the drug delivery application.¹⁷⁷

To achieve targeted drug delivery to minimize the side effect brought by nonspecific drug delivery, a folic acid–calix[4]arene conjugate was prepared (Figure 10a).¹⁷⁸ Folic acid is known to serve as an efficient cancer cell targeting ligand, capable of binding to the folic acid receptor that is usually overexpressed in most cancer cells. In this conjugate, four folic acid molecules were covalently coupled onto calix[4]arene via “click chemistry” at the upper rim, while four triethylene glycol moieties were introduced at the lower rim to improve the water solubility. The folic acid–calix[4]arene conjugate could effectively encapsulate a hydrophobic drug model indomethacin, and enhance its solubility in water. Although biological study was absent, such conjugation with the targeting moiety might bring a new diversity to CA-based drug delivery systems.

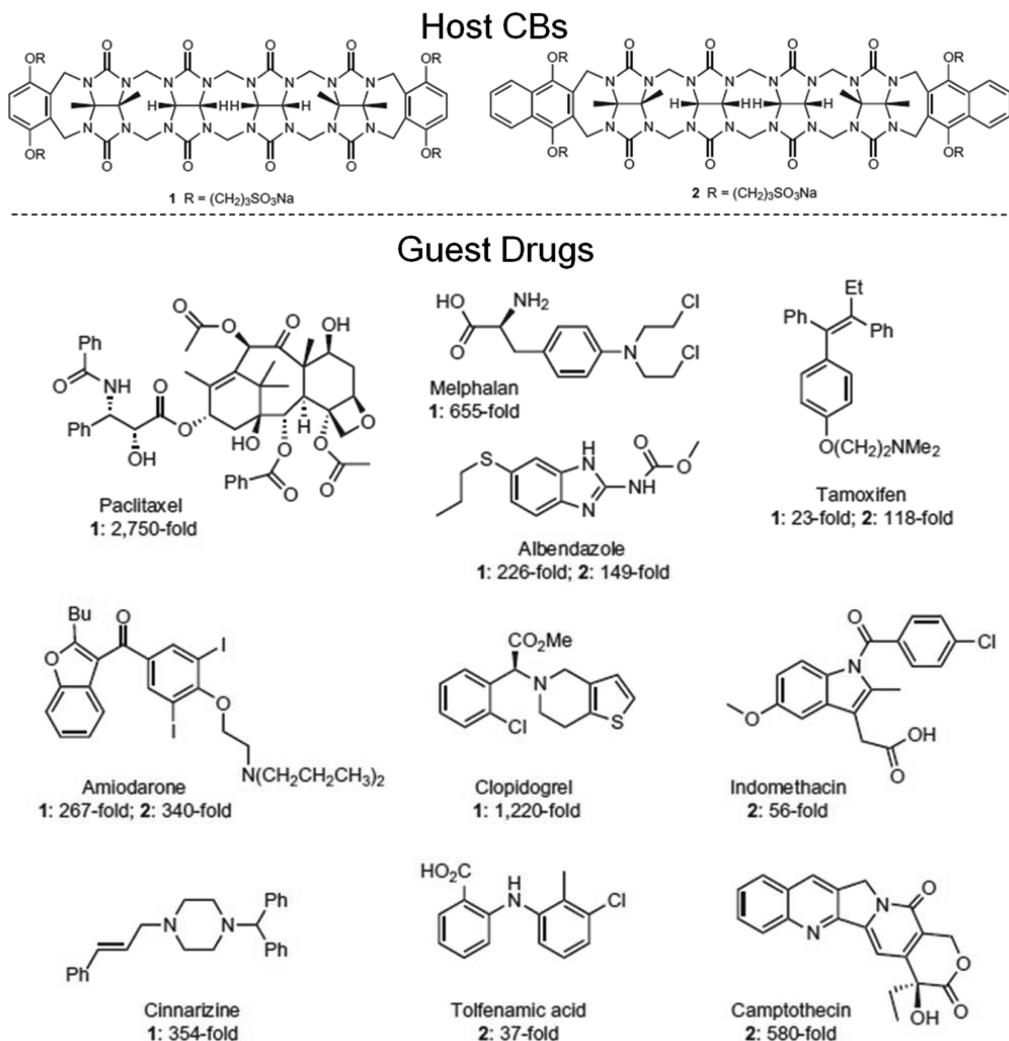


Figure 8. Acyclic CB[*n*] containers **1** and **2**. Phase solubility diagrams allow a determination of the enhancement in solubility for poorly soluble drugs in the presence of molecular containers **1** and **2**. Reprinted with permission from ref 166. Copyright 2012 Nature Publishing Group.

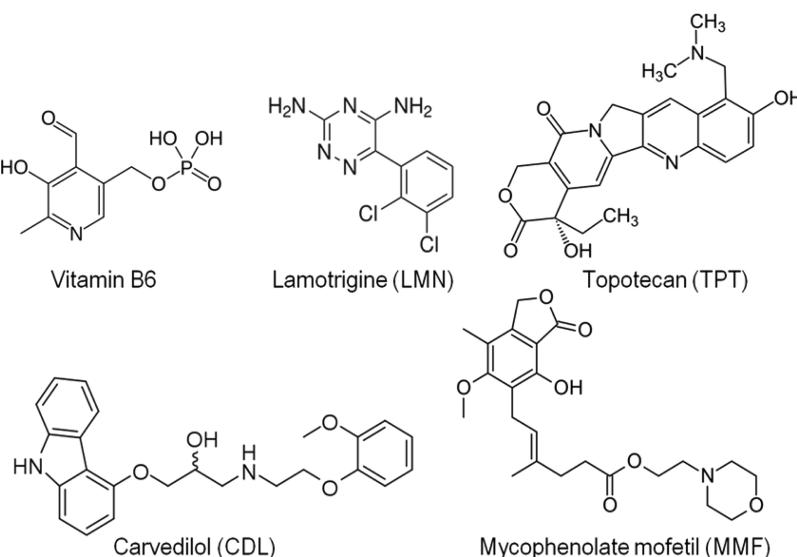


Figure 9. Various drugs used to form the host–guest complexes with *para*-sulphonatocalix[4]resorcinarene.

Strong host–guest inclusion could ensure the drug loading at high efficiency. On the other hand, high affinity might bring a

new problem for the drug release. Stimuli-responsive drug delivery can overcome this drawback. Xiao and co-workers

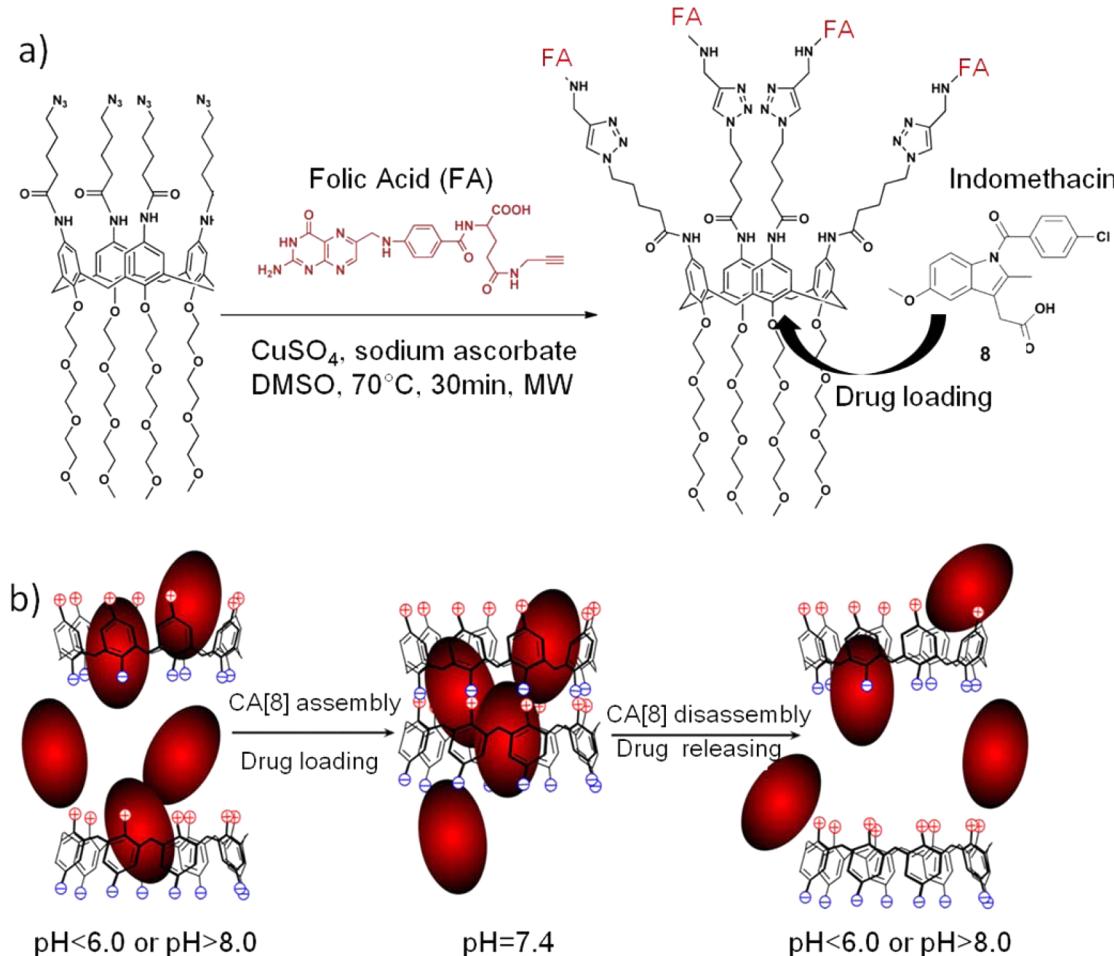


Figure 10. (a) Synthesis strategy of folic acid conjugated CAs for drug delivery by forming the host–guest inclusion with indomethacin. Reprinted with permission from ref 178. Copyright 2011 The Royal Society of Chemistry. (b) Schematic illustration of pH-triggered drug loading and release from amphoteric calix[8]arene (AC[8]). Reprinted with permission from ref 179. Copyright 2013 Elsevier.

designed and fabricated an amphoteric calix[8]arene (CA[8]) to achieve pH-responsive drug release from CA[8]–drug complex (Figure 10b).¹⁷⁹ The upper rim of the CA[8] was featured with negatively charged sulfonate groups, while the lower rim was grafted with positively charged quaternary ammonium groups. Hydrophobic model drug, ciprofloxacin (CPF), was loaded into the CA[8] cavity at neutral pH with the loading capacity of 17.8–24.5%. The CA[8]–CPF complex could self-assemble to form superstructures through the electrostatic interaction between the upper and lower rims. Under either acidic or basic pH condition, the CA[8]–CPF complex would disassemble and release the CPF drug in a sustained manner. Such pH-responsive drug delivery system shows great potential for future theranostic applications.

A summary of direct host–guest complexation for drug delivery applications is listed in Table 2. Using CD-based complexes for the drug delivery has been well established, and many products have been successfully commercialized. There was almost no significant development of CD-based simple host–guest inclusion complexes for drug delivery during recent years. Instead, more efforts have been devoted to CD-based supramolecular nanomaterials, aiming at the fabrication and application of multifunctional drug delivery systems. As compared to well-developed drug carriers based on CDs, supramolecular systems based on CBs and CAs have been relatively less studied for biomedical applications, due to their

inherent drawback of poor water solubility (like CAs) and short development history (like CBs). Moreover, CBs and CAs often require the postfunctionalization to achieve better water solubility and biocompatibility, which may lead to additional questions on the biosafety of the newly functionalized molecules. Thorough studies on their pharmaceutical effect are still under exploration, especially for the toxicity effect provided by the functional groups used to improve the water solubility of macrocyclic molecules. Nevertheless, an increasing number of publications in this field has clearly indicated great application potentials of these macrocyclic molecules in biomedical area.

2.1.2. Supramolecular Nanocarriers. In virtue of advantages of nanosized delivery systems, such as enhanced cellular uptake, controlled drug release, and targeted drug delivery, increasing attention has been paid to employing the nanocarriers in advanced drug delivery.^{180–182} Because of the superiority of using supramolecular chemistry approaches to construct nano-systems for drug delivery, a variety of supramolecular nanocarriers in the forms of micelles, vesicles, and supramolecular nanoparticles were developed. In particular, host–guest interaction has played a critical role in the preparation process of supramolecular drug nanocarriers. By integration with other nanomaterials, researchers have also developed many novel nanohybrids with multifunctional capability, aiming at the fabrication of a new generation of theranostic platforms. These

Table 2. Summary of Direct Host–Guest Complexation for Drug Delivery

host molecules	guest molecules	drug delivery	ref
β -CD and its derivatives, 2-hydroxypropyl- β -CD	delta-8-tetrahydrocannabinol	enhanced ocular bioavailability	152
β -CD	paclitaxel (PTX)	host–guest complex (β -CD:PTX = 1:1)-based hybrid and vesicle for drug delivery	153,154
CB[7]	pyrazinamide (pyrazine-2-carboxamide) and isoniazid (isonicotinohydrazide)	treatment of tuberculosis	155
CB[8]	fullerene[60]	in vitro antitumor activity against HeLa cells	156
CB[7]	triamterene	pharmacokinetics study indicated increased solubility and oral bioavailability	157
CB[7], CB[8]	cisplatin and multinuclear platinum complexes	encapsulation study and molecular modeling, reduced cytotoxicity, cytotoxicity study in L1210 cell line	133,158,159
CB[6], CB[7], CB[8]	albendazole and derivatives	aqueous solubility was increased by 2000-fold	131,132
CB[7], CB[8]	prototropicform of gefitinib	lung cancer treatment	160
CB[6], CB[7], CB[8], CB[10]	platinum(II)-based anticancer complexes and platinum(II)-based DNA intercalators	prevented drugs from degradation and reduced metal toxicity, cytotoxicity study for DNA intercalators	161,162
CB[7]	cisplatin	in vitro cytotoxicity study in carcinoma cell line A2780 and in vivo cytotoxicity study in human tumor xenografts	163
CB[n] (n = 6,7,8,9)	Pt-drugs (oxaliplatin, nedaplatin, carboplatin, and cisplatin)	complexing study by density functional theory	164
CB[7]	tomozolomide (TMZ)	chemotherapeutic study against glioblastoma multiforme (GBM)	165
CB[n] (n = 5,6,7,8,10)	paclitaxel (PTX), albendazole, tamoxifen, amiodarone, clopidogrel, indomethacin, cinnarizine, tolfennamic acid, camptothecin (CPT)	enhanced water solubility by a factor of between 23 and 2,750, in vitro toxicity study in HEK 293, HepG2, and THP-1 cells	166
CB[n] (n = 5,6,7,8,10) derivatives	curare-type neuromuscular blocking agents (NMBAs)	neuromuscular block in vivo	167
CB[n] (n = 5,6,7,8,10) with varied solubilizing groups (SO_3^- , OH, NH_3^+)	cationic (tamoxifen), neutral (17 α -ethynylestradiol), or anionic (indomethacin) drugs	complexing activity study	168
biotin-functionalized CB[7]	albendazole, tamoxifen, camptothecin (CPT), irinotecan, and temezolomide	in vitro study of targeted drug delivery toward L1210FR cells	169
p-sulphonatocalix[4]resorcinarene (PSC[4]R)	mycophenolate mofetil (MMF) and inosine monophosphate dehydrogenase (IMPDH)	water solubility enhancement and in vivo study with Swiss albino mice	170
PSC[n]R (n = 4 and 6)	carvedilol (CDL) and lamotrigine (LMN)	improved in vitro dissolution profile and decreased in vivo acute oral toxicity	171,173
sulfonatocalix[4]arene (SC4A)	topotecan (TPT)	complexing studies such as stoichiometry, complex stability constants, and inclusion mode	172
O-phosphorylated calix[n]arenes and P-phosphorylated CAs (n = 4, 6, and 8)	neutral molecules, such as nifedipine, niclosamide, and furosemide	complexing and solubility study	174,175
achiral CAs[n] and derivatives (n = 4, 6, and 8)	chiral pharmaceutical reagents	complexing study by circular dichroism	176
p-sulfonatocalix[6]arene	vitamin B6	complexing study by ^1H NMR technique	177
folic acid-calix[4]arene conjugate	hydrophobic drug model indomethacin	targeted drug delivery by enhanced drug solubility	178
amphoteric calix[8]arene (CA[8])	hydrophobic model drug, ciprofloxacin (CPF)	pH-responsive drug release	179

promising supramolecular nanocarriers are discussed in this section.

2.1.2.1. Vesicles. From the view of cell biology, a vesicle is composed of lipid bilayer membrane inside cells. Because of uniform nanoscale size, ease of fabrication, and empty hollow cores for a large amount of drug storage, plenty of vesicles have been developed for drug delivery. Supramolecular chemistry approaches have been long utilized to prepare artificial vesicles by noncovalent interactions,¹⁸³ such as polar/nonpolar interaction provided by amphiphilic surfactants.¹⁸⁴ Inside the core of formed vesicles, hydrophobic or hydrophilic drugs can be readily loaded for the purpose of drug delivery.¹⁸⁵

Amphiphilic molecule is usually needed for the formation of vesicles. The functions of the host–guest inclusion in the vesicle formation include the fabrication of supramolecular amphiphiles, acting as the driving force for the vesicle formation, as well as providing facile modifications on the external surface of vesicles. For instance, to achieve amphiphilic property for macrocyclic molecules, water-soluble tetraethylene glycol (TEG) was chemically conjugated onto the upper rim of thioether cavitand, while the lower rim was featured with hydrophobic hydroxyl chains. The formed vesicles not only exhibited high water

solubility, but also were capable of hosting different guest molecules via the host–guest interactions (Figure 11a).¹⁸⁶ Ravoo and co-workers functionalized long hydrophobic alkyl tails onto one side of β -CD, yielding amphiphilic β -CD that could form vesicles. The hydrophobic alkyl tails were directed inward, while hydrophilic hydroxyl groups of β -CD stretched into the external aqueous environment. Maltose and/or lactose was then modified on the vesicle surface via the host–guest inclusion between β -CD and adamantane (Ad) unit from Ad-conjugated maltose and/or lactose.^{187–189} Pseudorotaxane formed by the host–guest interaction between CB[6] and N,N'-hexamethylenebis(1-octyl-4-carbamoylpyridinium bromide) (HBPB-8) was prepared by threading CB[6] onto the HBPB-8 chain that has two hydrophobic ends. During the vesicle formation, the hydrophilic CB[6] units face outside toward the aqueous environment, while the two hydrophobic ends of HBPB-8 face inward toward the core (Figure 11b).¹⁹⁰

In another case, hydrophilic hyperbranched polyglycerol (HPG) was covalently conjugated onto β -CD (CD-HPG), and hydrophobic alkyl chain (C₁₈) was coupled with Ad molecule (Ad-C₁₈). Through the host–guest complexation between β -CD and Ad, supramolecular amphiphiles were constructed, which

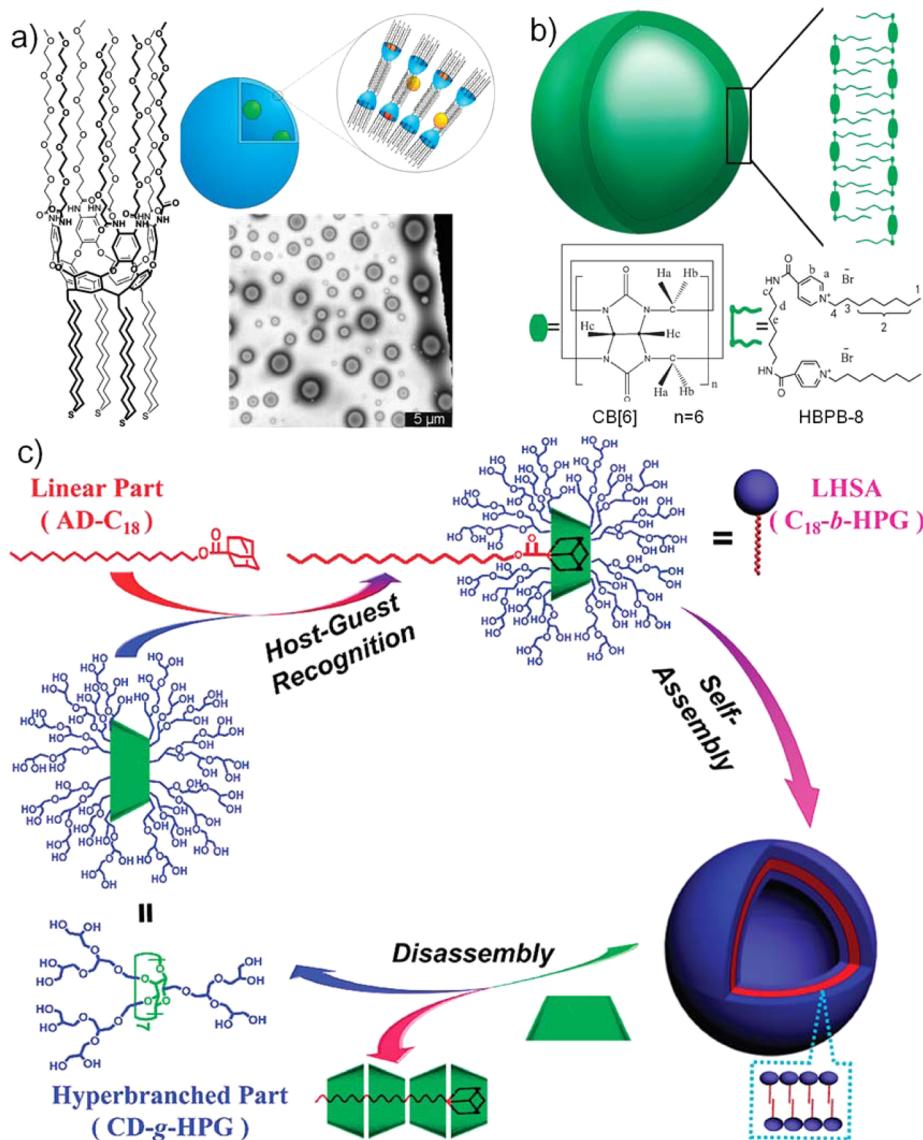


Figure 11. (a) Schematic representation of an idealized deep cavitand vesicle with a bilayer structure consisting of interdigitated packing of alkyl chains and TEG groups facing water inside and outside the vesicle. Inset is a TEM image of vesicles. Reprinted with permission from ref 186. Copyright 2012 The Royal Society of Chemistry. (b) Structures of supramolecular vesicle constructed by host CB[6] and guest HBPPB-8. Reprinted with permission from ref 190. Copyright 2011 The Royal Society of Chemistry. (c) Preparation, self-assembly, and disassembly of the vesicle by a linear hyperbranched supramolecular amphiphile. Reprinted with permission from ref 191. Copyright 2011 American Chemical Society.

could further self-assemble into core–shell structured vesicles (Figure 11c).¹⁹¹ Similarly, the host–guest interaction between β -CD and Ad was utilized to induce the formation of polymer vesicle aggregates.¹⁹² The access to empty cavity of excess β -CD provides a possibility for the postmodification on the vesicle surface via the host–guest complexation. Researchers also used hydrophobic polymer polystyrene (PS) with aromatic units as the backbone for the vesicle formation. The host–guest interaction between the CD–HPG conjugate and the aromatic units could lead to the formation of supramolecular vesicles in aqueous solution. By completing the self-assembly process in solution containing anticancer drug PTX, the vesicles could successfully load PTX into its cores and then serve as hydrophobic drug delivery carriers. The PTX-loaded vesicles exhibited pH-dependent release property with high release rate under acidic environment.¹⁹³

Kim and co-workers developed two different approaches for the vesicle preparation. In 2005, they reported the vesicle preparation by amphiphilic CB[6] derivatives, where the periphery was functionalized with triethylene glycol groups. By utilizing facile host–guest complexation between hydrophobic cavity of CB[6] and polyamine, the surface of the vesicles could be easily modified with molecular tags.¹²⁵ Afterward, they introduced redox-responsive disulfide unit into the functional groups at the periphery of amphiphilic CB[6], resulting in a reduction-sensitive vesicle (Figure 12).¹⁹⁴ Polyamine conjugated folic acid or fluorescence molecule (such as fluorescein isothiocyanate, FITC) was introduced onto the vesicle surface through the host–guest complexation for cancer-targeted drug delivery and fluorescence bioimaging. Anticancer drug doxorubicin (Dox) was loaded inside the vesicles, which could be effectively delivered into HeLa cancer cells through folic acid receptor-mediated endocytosis of the multifunctional vesicles. In

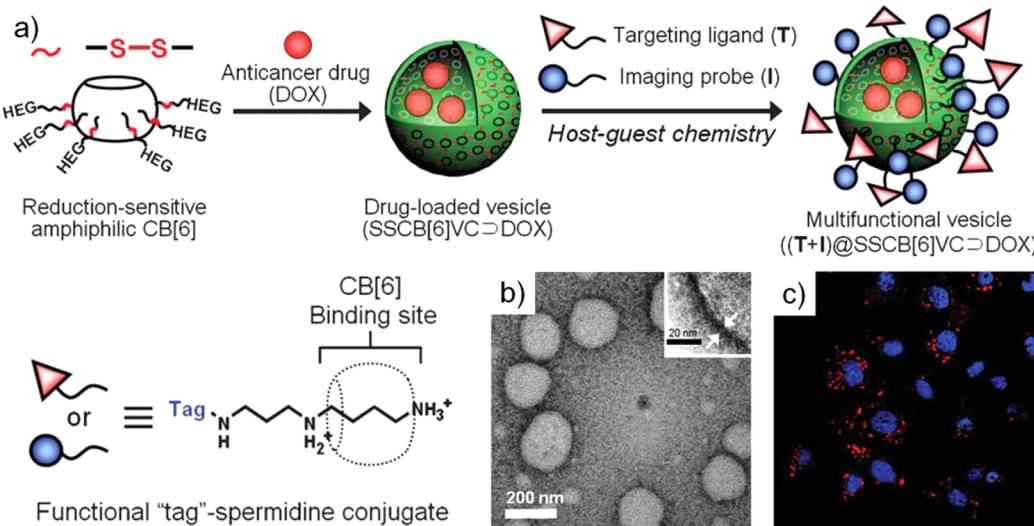


Figure 12. (a) Reduction-sensitive CB[6]-based vesicle (SSCB[6]VC) with noncovalently modifiable surface and its application as a multifunctional platform for targeted drug delivery (HEG: hexaethylene glycol). (b) TEM image of SSCB[6]VC, and (c) confocal laser scanning microscopy image of HeLa cells incubated with doxorubicin (Dox) loaded SSCB[6]VC. Adapted from ref 194. Copyright 2010 Wiley-VCH Verlag.

another approach, they utilized thiol–ene “click” reaction to directly connect dithiols with CB[6] derivatives bearing allyloxy groups at the periphery, affording robust polymer nanocapsules.¹⁹⁵ Similarly, they later embedded disulfide bond into the functional groups at the periphery of CB[6] derivatives to form reduction-responsive polymer capsules. Polyamine conjugated cancer cell targeting ligand was introduced onto the capsule surface via the host–guest interaction. The multifunctional reduction-responsive polymer capsules show targeted delivery of carboxyfluorescein (CF) toward HepG2 liver cancer cells.¹⁹⁶

As controlled drug delivery can greatly minimize side effects generated from the drug prerelease before reaching the target cells, it is scientifically necessary to fabricate responsive drug delivery vesicles. In 2011, Liu and co-workers reported a multi stimuli-responsive supramolecular binary vesicle by the host–guest complexation between a water-soluble CA, *p*-sulfonatocalix[4]arene (C4AS), and asymmetric viologen, 1-methyl-10-dodecyl-4,40-bipyridinium (MVC12) (Figure 13a).¹⁹⁷ Anticancer drug Dox was then loaded into the vesicle. The enthalpy-driven complexation between C4AS and MVC12 endowed the thermal responsiveness. Tunable molecular amphiphilicity of MVC12 was controlled by either the redox potential or the inclusion of CDs. As a result, the delivery system presented multiresponsive Dox release properties. In vitro study revealed the feasibility of the drug delivery system in the cancer treatment. Afterward, they further developed an enzyme-responsive supramolecular vesicle by employing biocompatible *p*-sulfonatocalix[4]arene (SC4A) to deliver tacrine specifically for the treatment of Alzheimer’s disease.¹⁹⁸ The binary vesicle was formed by the host–guest complexation between SC4A and myristoylcholine. The vesicle exhibited highly specific and efficient responsiveness to cholinesterase enzyme that could break the hydrophilic–hydrophobic balance, leading to the disassembly of the binary vesicle and thus the release of loaded drugs. Such kind of binary vesicles could be achieved using other host molecules (Figure 13b). In 2013, Wang and co-workers managed to construct a novel type of supramolecular vesicles with a thin thickness of about 7 nm, where the self-assembly process was driven by the host–guest inclusion between a water-

soluble pillar[6]arene (WP6) and hydrophobic ferrocene derivative, hydrophobic N-1-decylferrocenylmethylamine (G), in aqueous environment (Figure 13c).¹⁹⁹ Anticancer drug mitoxantrone (MTZ) was encapsulated in the vesicles. The rapid MTZ release under acidic pH indicates the application potentials of the vesicle carriers for controlled drug release inside the cancer cells. The drug delivery system was tested using SMMC-7721 cancer cell and NIH3T3 normal cell, respectively. Very recently, Wang and co-workers reported another multiple stimuli-responsive supramolecular vesicle by using WP6. Anticancer drug Dox was loaded and then released in a controllable manner.²⁰⁰

2.1.2.2. Micelles. Micelle is another important type of supramolecular self-assembly that can be used for drug delivery. Typically, it is formed by amphiphilic surfactant that possesses both hydrophobic “heads” facing the surrounding environment and hydrophobic tails heading toward the micelle center. For example, amphiphilic block copolymers with hydrophilic and hydrophobic sections at two ends have been widely utilized for the micelle preparation.²⁰¹ Amphiphilic CAs and resorcinarenes can either form vesicles or micelles, and the formation mechanism of micelles is similar to that of vesicles.²⁰² Because of the biocompatibility and stimuli-responsive degradation capability, a variety of micelles fabricated on the basis of the host–guest complexation have been developed for the drug delivery.

Dong and co-workers fabricated micelles on the basis of a biodegradable block copolymer, where the host–guest inclusion between β -CD and polymer chain induced the transition from micelles to hydrogels that are a popular material for injectable drug delivery and tissue engineering. The developed micelle-hydrogel drug delivery system presented reduction-responsive Dox release on account of pre-embedded disulfide bond in the block copolymer main chain.²⁰³ In addition to redox-responsive drug release from micelles, thermal-responsive micelles based on temperature-sensitive block copolymer composed of *N*-isopropylacrylamide (NIPAAm) and trimethylsilylpropargyl acrylate (TMSPA) were prepared for albendazole (ABZ) delivery in vitro. The β -CD ring was functionalized onto NIPAAm as the pendant to endow the block copolymer with

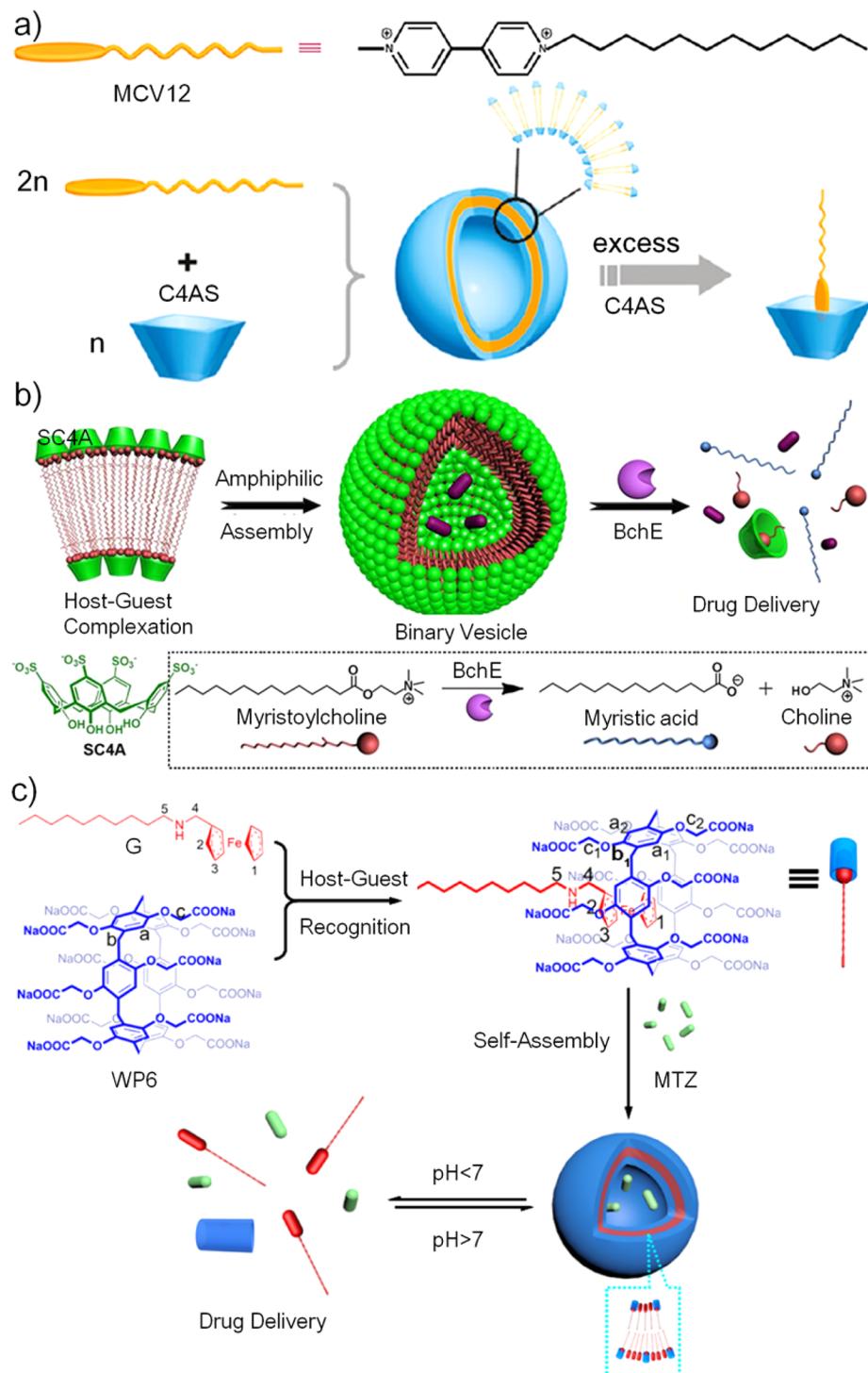


Figure 13. (a) Schematic illustration for the construction of a supramolecular binary vesicle based on the host–guest complexation of C4AS with MCV12. Reprinted with permission from ref 197. Copyright 2011 American Chemical Society. (b) Amphiphilic assembly of myristoylcholine in the presence of SC4A for responsive drug release. Reprinted with permission from ref 198. Copyright 2012 American Chemical Society. (c) Formation of a supramolecular vesicle and its pH-responsive drug release. Reprinted with permission from ref 199. Copyright 2013 American Chemical Society.

amphiphilic property, subsequently leading to the micelle formation by self-assembly in aqueous environment. Another role of β -CD here was to encapsulate the ABZ drug via the host–guest interaction.²⁰⁴

By using a different approach, supramolecular capsules were prepared from the polymers that were covalently conjugated with functionalized α -CD as a pendant. Carboxymethyl dextran grafted α -CD (CMD-g- α -CD) and poly(acrylic acid) grafted α -CD (PAA-g- α -CD) were first synthesized. The host–guest inclusion between α -CD and azobenzene unit assisted the capsule formation on the surface of solid CaCO_3 nanoparticles acting as a solid template. After the removal of CaCO_3 , supramolecular hollow capsules were obtained. The hollow capsules were responsive to UV light irradiation, which could trigger the disassociation of α -CD-azobenzene host–guest complex for remote controlled drug

dodecane *p*-azobenzene aminosuccinic acid (PAA-C12-Azo) were first synthesized. The host–guest inclusion between α -CD and azobenzene unit assisted the capsule formation on the surface of solid CaCO_3 nanoparticles acting as a solid template. After the removal of CaCO_3 , supramolecular hollow capsules were obtained. The hollow capsules were responsive to UV light irradiation, which could trigger the disassociation of α -CD-azobenzene host–guest complex for remote controlled drug

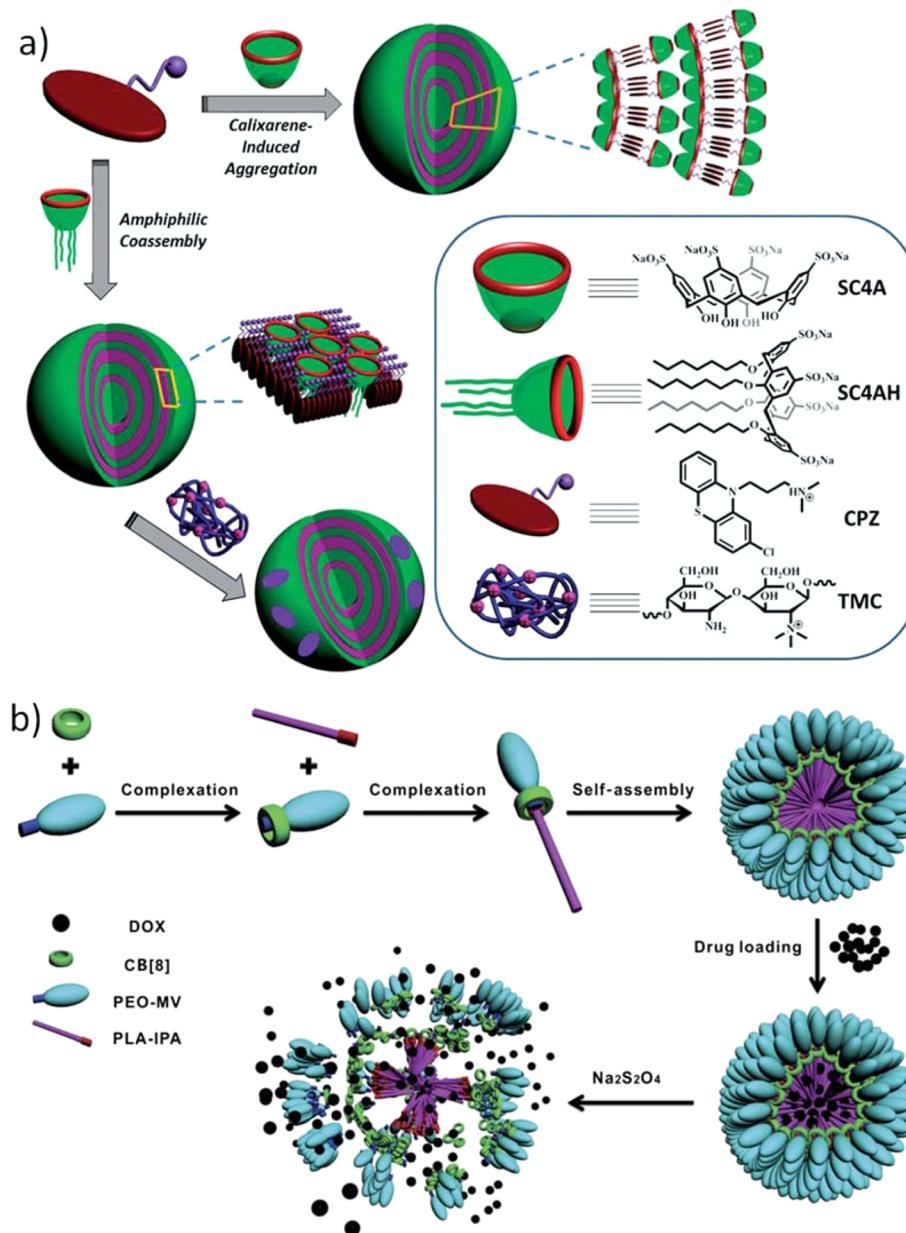


Figure 14. (a) Micelle formation by coassembly of an amphiphilic drug with CAs. Reprinted with permission from ref 207. Copyright 2014 The Royal Society of Chemistry. (b) Formation, drug loading, and reduction-triggered drug release of CB[8]-based supramolecular micelles. Reprinted with permission from ref 208. Copyright 2014 The Royal Society of Chemistry.

delivery.²⁰⁵ Later, the same research group utilized this solid template approach to fabricate pH-responsive supramolecular capsules by introducing a pH-sensitive C=N bond into the system. In vitro study using HeLa cells under different pH conditions revealed the pH controlled release property of the capsules, indicating the application potentials as pH-responsive drug delivery carriers.²⁰⁶

Very recently, Liu and co-workers reported supramolecular multilayer spherical micelles formed by direct assembly of the host–guest complex between small antipsychotic drug chlorpromazine (CPZ) and *p*-sulfonatocalix[4]arene (SC4A) or tetraheptyl ether conjugated *p*-sulfonatocalix[4]arene (SC4AH) (Figure 14a). The system showed the drug loading efficiency as high as 61% and 46% for the two types of micelles. A targeting ligand trimethylated chitosan (TMC) could be readily modified onto the external layer of the micelles via the host–

guest inclusion with hydrophobic cavity of SC4AH, providing another advantage for serving as a versatile drug carrier.²⁰⁷ By introducing adenosine triphosphate (ATP) as a guest molecule to form the host–guest complex with an amphiphilic CA (AC4AH), phosphatase-responsive supramolecular micelles were obtained via the self-assembly. The phosphatase-triggered hydrolysis of ATP could lead to the dissociation of the supramolecular micelles, causing the drug release from the micelles. When considering the overexpression of phosphatase in tumor cells, this type of micelles exhibits great potentials for triggered drug delivery in the cancer treatment.²⁰⁸

Direct host–guest interaction between one macrocyclic molecule and two guest molecules with different properties (such as hydrophobic and hydrophilic) can yield a ternary complex as an amphiphile for the micelle construction. Ji and co-workers used CB[8] to directly host two amphiphilic unites, that

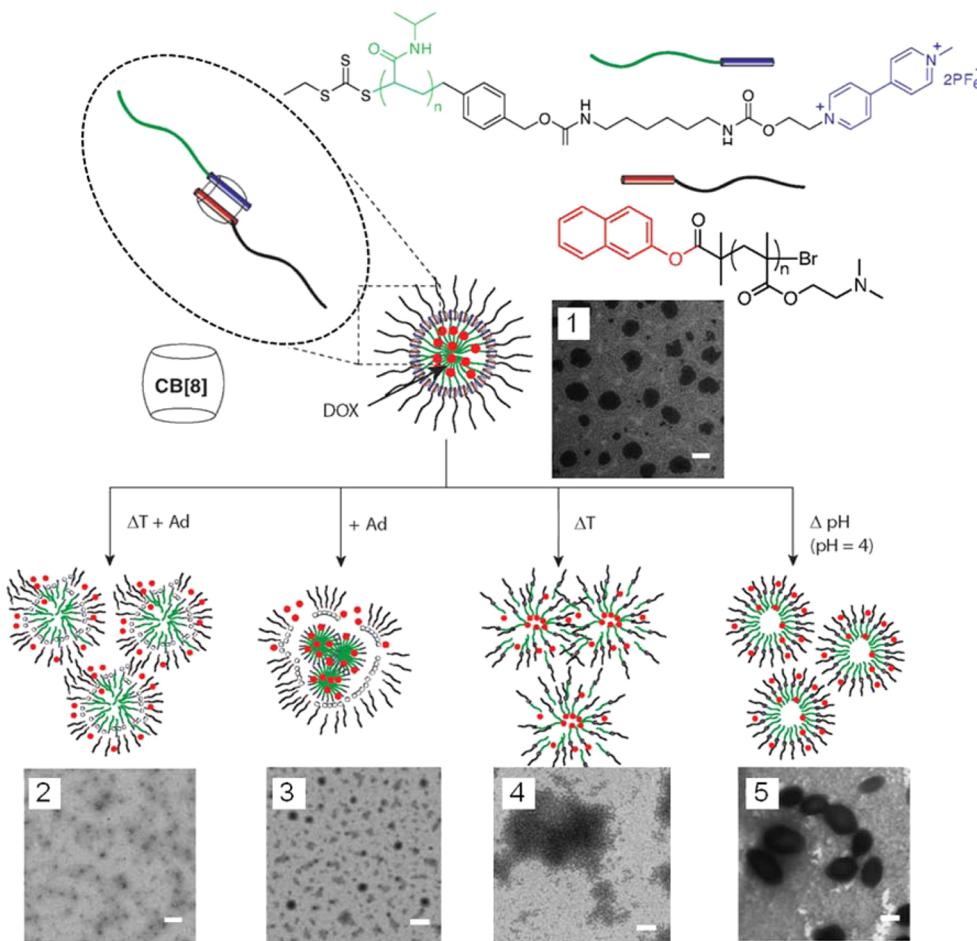


Figure 15. Hierarchical self-assembly of the supramolecular entity under different conditions and its subsequent mode of drug release after being exposed to different triggers. Insets 1–5 are corresponding TEM images of the micelles before and after exposure to various stimulus. Reprinted with permission from ref 211. Copyright 2011 American Chemical Society.

is, methyl viologen-containing hyperbranched polyphosphate (HPHEEP-MV) and indole-terminated poly(D,L-lactide) (PLA-IPA), forming a ternary complex noted as HPHEEP-CB[8]-PLA. The amphiphilic ternary complex could form micelles with PLA facing inside and HPHEEP facing outside. Upon the addition of Ad or $\text{Na}_2\text{S}_2\text{O}_4$, the formed micelles could be destructed by competitive binding into the cavity of CB[6], resulting in the release of loaded hydrophobic drug coumarin 102.²¹⁰ By employing CB[8], PLA-IPA, and methyl viologen-terminated poly(ethylene oxide) (PEO-MV), a supramolecular amphiphilic complex was prepared, which could further self-assemble into the micelles within a nanoscale range. Anticancer drug Dox was loaded into the micelles, and its release could be triggered by the addition of $\text{Na}_2\text{S}_2\text{O}_4$, as $\text{Na}_2\text{S}_2\text{O}_4$ could effectively form a host–guest complex with CB[8] to replace PLA-IPA and PEO-MV to result in the dissociation of supramolecular micelles. In vitro cell viability study indicated good biocompatibility of the micelles toward two cell lines, that is, human umbilical vein endothelial cell line (HUVEC) and human liver cancer HepG2 cell line. Enhanced toxicity of Dox against human liver cancer HepG2 cell line was observed (Figure 14b).²⁰⁸ Moreover, Loh and co-workers also prepared a ternary complex between CB[8] and two hydrophilic block copolymers, that is, naphthalene-terminated poly(dimethylaminoethyl methacrylate) (PDMAEMA) and methylviologen terminated poly(N-isopropylacrylamide) (PNIPAM) (Figure 15).²¹¹ The two polymers are responsive to pH

and temperature, respectively. The ternary complex constructed by CB[8], PDMAEMA, and PNIPAM could further form micelles, which could encapsulate anticancer drug Dox within the micelles. In addition to the pH and temperature control, the micelles were also responsive to competitive guest such as Ad. Thus, the system exhibited triple stimuli-responsive drug release properties. In another case, a ternary complex formed from CB[8] as well as temperature- and glucose-responsive building blocks, hydrophilic poly(nisopropylacrylamide) (PNIPAAm) and poly(acrylamidophenyl boronic acid) (PAAPBA), could self-assemble to construct the micelles capable of insulin delivery. The micelles were also responsive to three external stimuli, including the temperature change, the addition of glucose, and competitive guest molecules, all of which could effectively trigger the micelle dissociation and insulin release for diabetes treatment.²¹²

Mechanically interlocked molecule-based micelles can be formed by spontaneous assembly under controlled conditions for controlled drug delivery. Researchers studied the effects of pH and temperature upon the micelle formation from poly(ethylene oxide)-block-poly(acrylic acid)/ α -CD polypseudorotaxane.²¹³ Recently, Khashab and co-workers developed pH-responsive micelles by using a polypseudorotaxane based on CB[7] and polystyrene-block-polyvinylpyridine (PS-*b*-P4VP). The complexation of CB[7] under acidic or neutral pH condition could effectively slow the release of loaded hydrophobic cargo

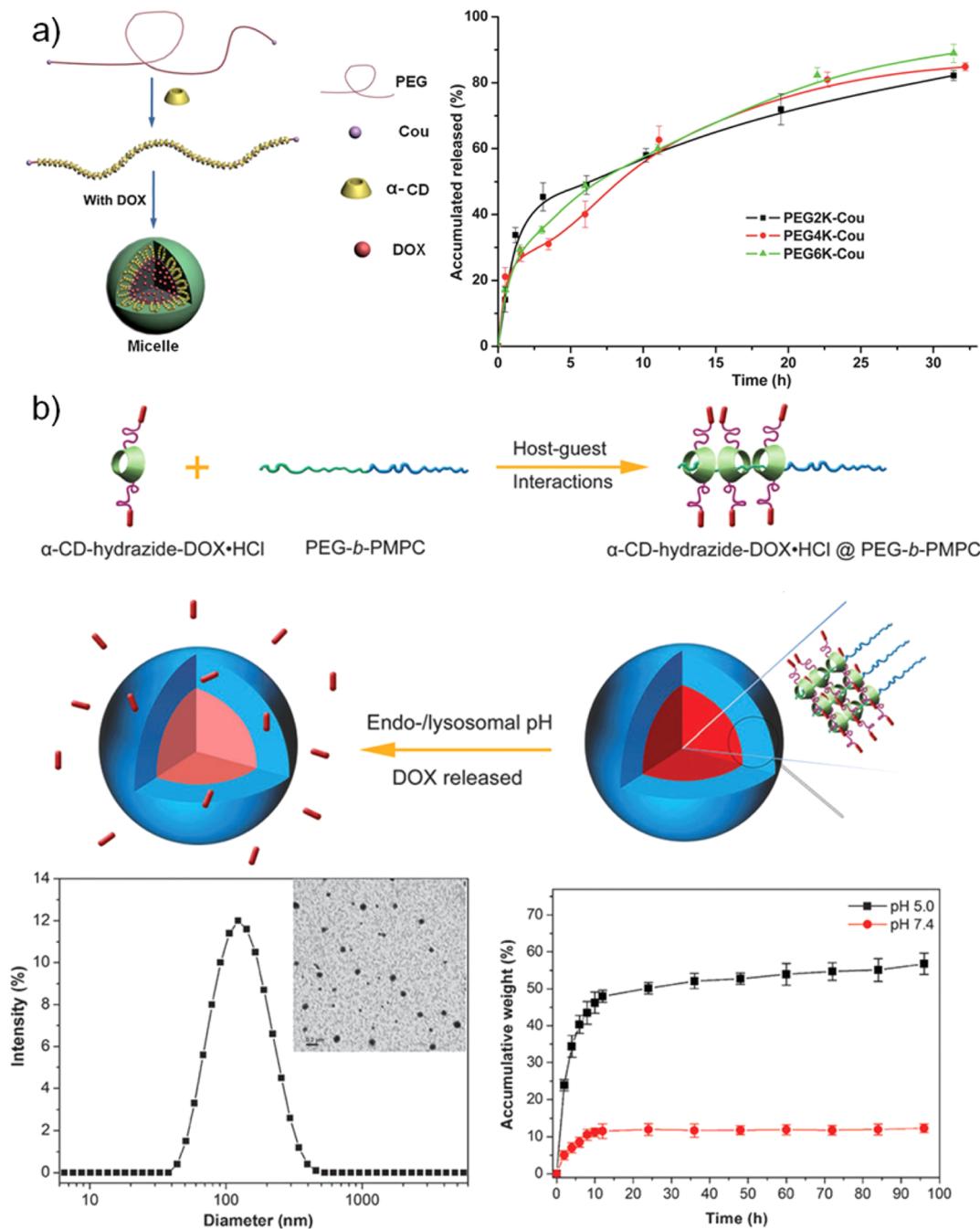


Figure 16. (a) Illustration for the fabrication of the Dox loaded polypseudorotaxane micelle (left) and the Dox release profiles (right). Reprinted with permission from ref 215. Copyright 2012 The Royal Society of Chemistry. (b) Schematic illustration for the preparation of supramolecular prodrug micelle and controlled release of Dox under endo/lysosomal pH stimulus (up), DLS plot and representative TEM image (bottom left) of the supramolecular micelle, and in vitro Dox release from the supramolecular prodrug micelle in PBS under different pH conditions (bottom right). Reprinted with permission from ref 217. Copyright 2013 The Royal Society of Chemistry.

model, pyrene. At basic pH condition, however, the dissociation of CB[7] from uncharged P4VP could lead to significant drug release.²¹⁴ Gu and co-workers also reported polypseudorotaxane-based micelles, in which α-CD was threaded onto hydrophobic coumarin terminated PEG polymer chain. The micelle formation was driven by hydrophobic interaction and poly-pseudorotaxane crystallization. Sustained release of trapped Dox could last up to 32 h. Effective cellular uptake of the supramolecular micelles and Dox release inside cancer cells were observed by CLSM. The Dox loaded micelles exhibited enhanced chemotherapy effect against B16 melanoma cells as

compared to free Dox (Figure 16a).²¹⁵ They also used cinnamic acid modified PEG to generate a polyrotaxane and then form supramolecular vesicles, which could be further converted into micelles after loading Dox. This drug delivery system was utilized for mouse 4T1 breast cancer treatment. Both in vitro and in vivo studies were carried out, indicating effective cancer-killing capability. Tumor growth inhibition rate of the Dox loaded micelles was 53%, higher than the case only treated with free Dox. Furthermore, cardiac toxicity of Dox was significantly decreased by encapsulating Dox into the supramolecular micelles.²¹⁶ Toward biomedical application of micelles, polypseudorotax-

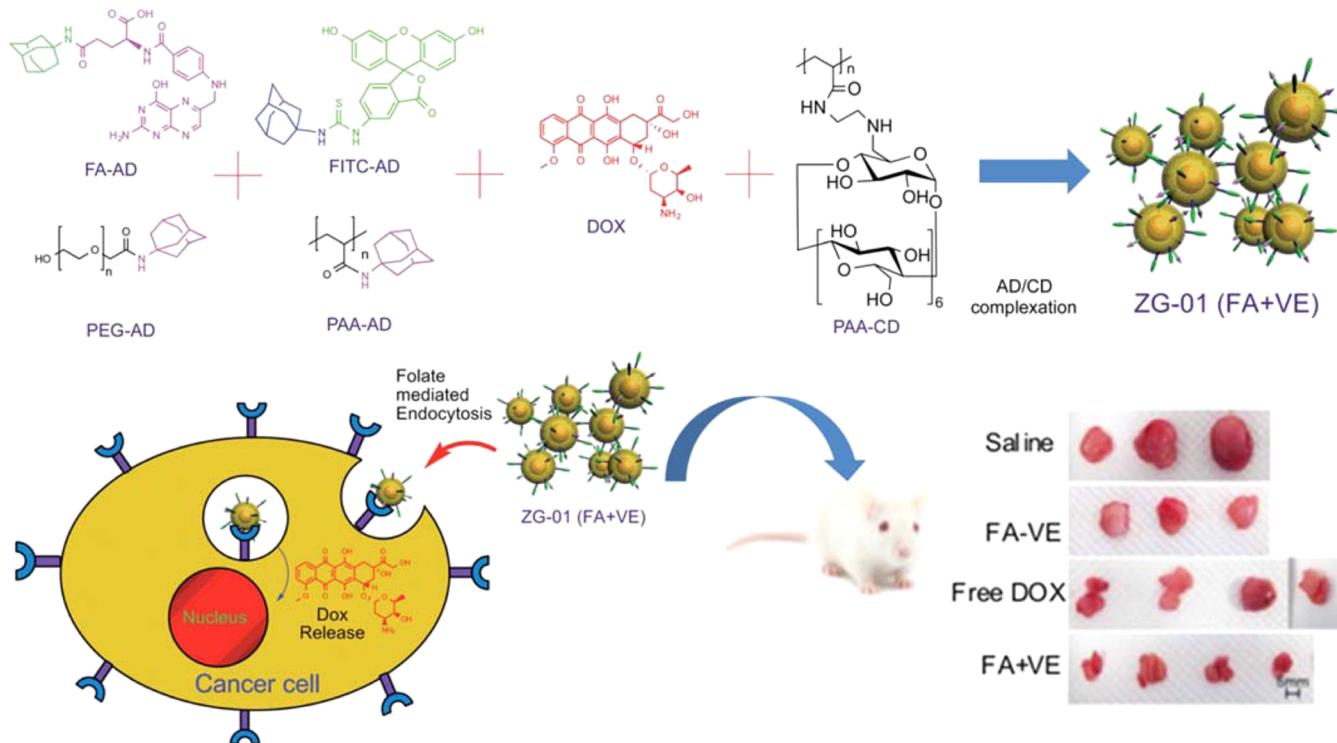


Figure 17. Formation of ZG-01 (FA+VE) nanoparticles through supramolecular self-assembly for target-specific drug delivery in cancer therapy. Adapted from ref 227. Copyright 2014 The Royal Society of Chemistry.

ane-based micelle prepared from CD and a block copolymer poly(ethylene glycol)-*b*-poly(2-methacryloyloxyethyl phosphorylcholine) (PEG-*b*-PMPC) was reported (Figure 16b).²¹⁷ The two hydrophilic precursors were mixed in aqueous solution, forming polypseudorotaxane-based micelle with the CD rings threaded onto the PEG segment of the copolymer. Anticancer drug Dox was loaded inside the micelle. The loaded Dox could be released under endosomal/lysosomal pH, which was observed in HepG2 liver cancer cell line by confocal laser scanning microscopy (CLSM).

2.1.2.3. Supramolecular Nanoparticles. Nanoparticles have enjoyed a rising popularity in the scientific community due to their special properties brought by the size effect. In biomedical applications, supramolecular particles within a nanoscale size have been developed for intracellular drug delivery. For instance, Thiele and co-workers used electrostatic interaction between anionic starch and cationic CD derivatives to form nanoparticles for targeted drug delivery. Pteroic acid was conjugated onto the nanoparticles as a cancer targeting ligand. Hydrophobic drug model, 1,4-dihydroxyanthraquinone (DHA), was loaded into the supramolecular nanoparticles by the host–guest complexation into the cavity of CD.²¹⁸

2.1.2.3.1. Rotaxane and Polyrotaxane-Based Supramolecular Nanoparticles. Rotaxane and polyrotaxane have been widely studied for biomedical applications. In previous content, we discussed the usage of poly(pseudo)rotaxanes for supramolecular micelle preparation and applications. In fact, polyrotaxanes could be directly used as the drug delivery carriers.²¹⁹ In early studies, researchers prepared polyrotaxanes by threading α -CD onto PEG polymer chains for anticancer drug Dox and CPT delivery. By conjugating cell-penetrating peptide (low molecular weight protamine) onto the polyrotaxane drug delivery system, improved cellular uptake of the polyrotaxanes into cancer cells and enhanced chemotherapy efficacy were

achieved.^{220,221} Through the integration of polyrotaxanes with some nanoparticles, such as quantum dots (QDs) and gold nanoparticles (AuNPs), drug delivery systems with new functions were prepared.^{222,223} Another popular approach is self-assembly of polyrotaxanes to form supramolecular nanoparticles for the purpose of drug delivery. For example, α -CD/PEG polyrotaxane-based supramolecular nanoparticles with a size around 200 nm were prepared by the self-assembly, and anticancer drug methotrexate (MTX) was loaded into the nanoparticles via hydrogen-bonding interaction. The formation mechanism, morphology, and drug release property of the MTX loaded supramolecular nanoparticles were investigated. In vitro cell study indicated that the polyrotaxane-based supramolecular nanoparticles were nontoxic. Enhanced anticancer capability was demonstrated by using MTX loaded nanoparticles in comparison with free MTX.²²⁴ Das and co-workers reported a polymer complex prepared by hydrogen-bonding interaction between two polymers, poly(acrylamide) (PAAm) and poly(vinyl alcohol) (PVA), for promising therapeutic application. The introduction of CD threaded onto linear polymer PAAm afforded polypseudorotaxane, which could further form supramolecular nanoparticles through the self-assembly.²²⁵ The supramolecular nanoparticles showed an enhanced antibacterial efficacy and long-lasting capability in bacterial imaging.

2.1.2.3.2. Host–Guest Interaction-Induced Supramolecular Nanoparticles. In addition to employing the poly(pseudo)-rotaxanes, the formation of supramolecular nanoparticles can be driven by the host–guest complexation between macrocyclic molecules and guest molecules, which are covalently conjugated on different polymer chains or other building blocks. During the formation process of supramolecular nanoparticles, anticancer drugs can be encapsulated or embedded inside the nanoparticles. The loaded drugs can present either sustained release from the supramolecular nanoparticles or controlled release by stimuli-

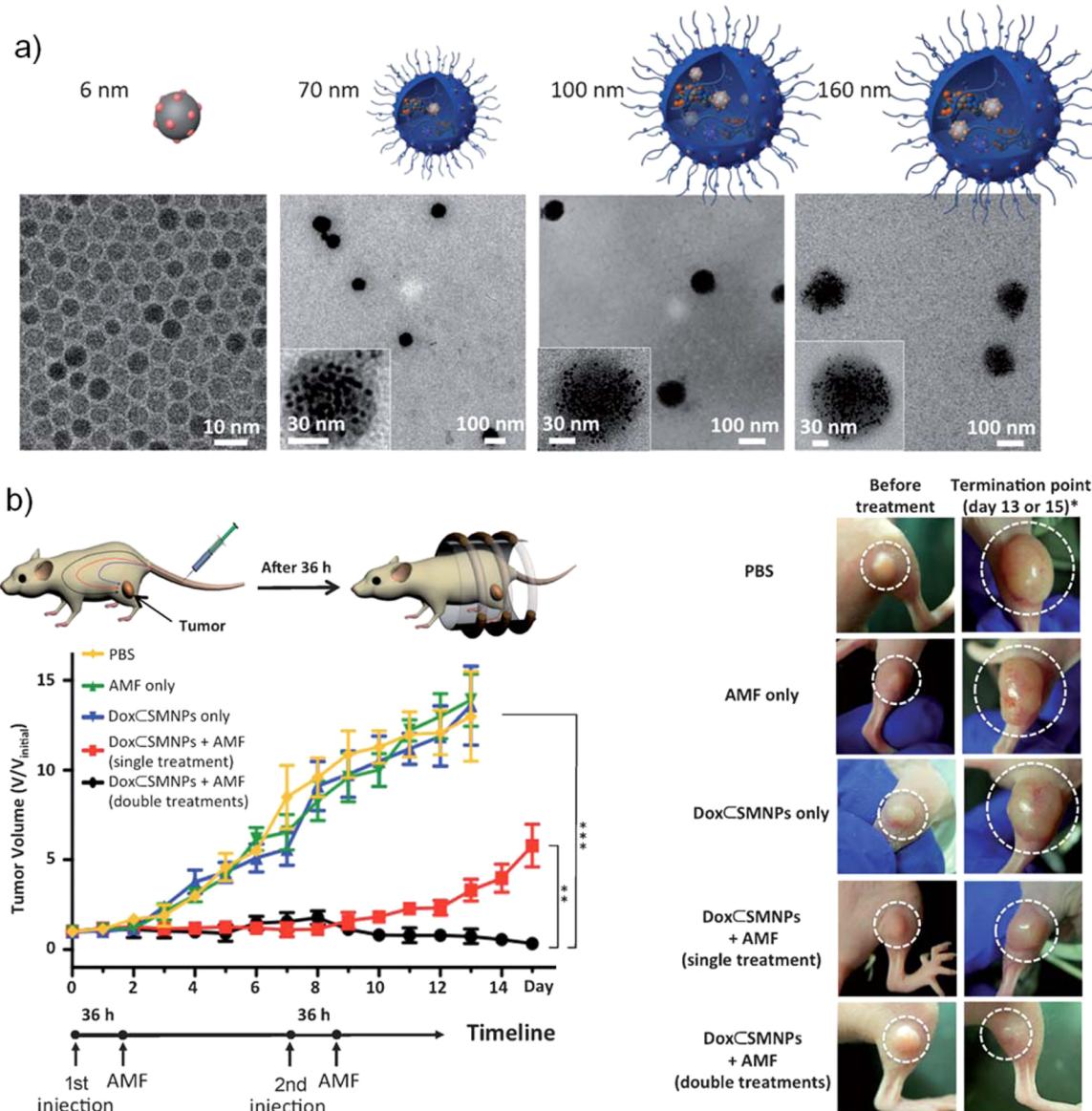


Figure 18. (a) TEM images of 6 nm Ad-MNP and Dox loaded SMNPs with various sizes of 70, 100, and 160 nm. (b) Evaluation of in vivo therapeutic efficacy. Treatment scheme of Dox loaded SMNPs in mouse, and the results of the tumor volume change over the course of the treatment (15 days) in DLD-1 (colorectal adenocarcinoma cell line) xenografted mice ($n = 3$) treated with Dox-loaded SMNPs (with and without the application of AMF) and control groups (AMF only and PBS only). Reprinted with permission from ref 234. Copyright 2013 Wiley-VCH Verlag.

triggered dissociation of the nanoparticles. For example, Liu and co-workers reported a method to fabricate supramolecular polysaccharide nanoparticles by utilizing the host–guest interaction between CD and amino-Ad, and managed to delivery adamplatin prodrug for cancer therapy.²²⁶ Zhao and co-workers recently synthesized multifunctional supramolecular nanoparticles based on β -CD conjugated poly(acrylic acid) (PAA-CD), Ad conjugated PAA (PAA-Ad), and Ad conjugated PEG (PEG-Ad) (Figure 17). Through the host–guest complexation between the Ad unit and β -CD, the supramolecular nanoparticles with a uniform size about 35 nm were formed, during which process Dox could be loaded simultaneously. Cancer cell targeting ligand folic acid and fluorescence probe FITC were both introduced into the supramolecular nanoparticles via the host–guest complexation between β -CD and Ad-functionalized folic acid (FA-Ad) and FITC (FITC-Ad). In this study, normal cell HEK 293 was chosen as a negative control, and two cancer

cell lines, MDA-MB231 breast cancer cells and B16F10 skin cancer cells, were used as the positive controls. Selective drug delivery and cancer killing effect toward the two cancer cell lines were achieved. In vivo study using the nanoparticles showed good tumor growth inhibition in nude mice bearing tumors.²²⁷

Ma and co-workers also developed supramolecular nanoparticles by conjugating β -CD onto the polymer side chain as a pendant for drug delivery. A block copolymer containing hydrophilic PEG segment and polyaspartamide bearing β -CD units (PEG- β -PCD) was synthesized. The cavity of β -CD could encapsulate anticancer drugs via the host–guest complexation, and the block copolymer could self-assemble into core–shell supramolecular nanoparticles.²²⁸ They also reported temperature-responsive supramolecular nanoparticles based on a block copolymer containing β -CD-modified segment and PEG segment (PEG- β -PCD). The host–guest interaction between CD and the isopropyl group of poly(*N*-isopropylacrylamide)

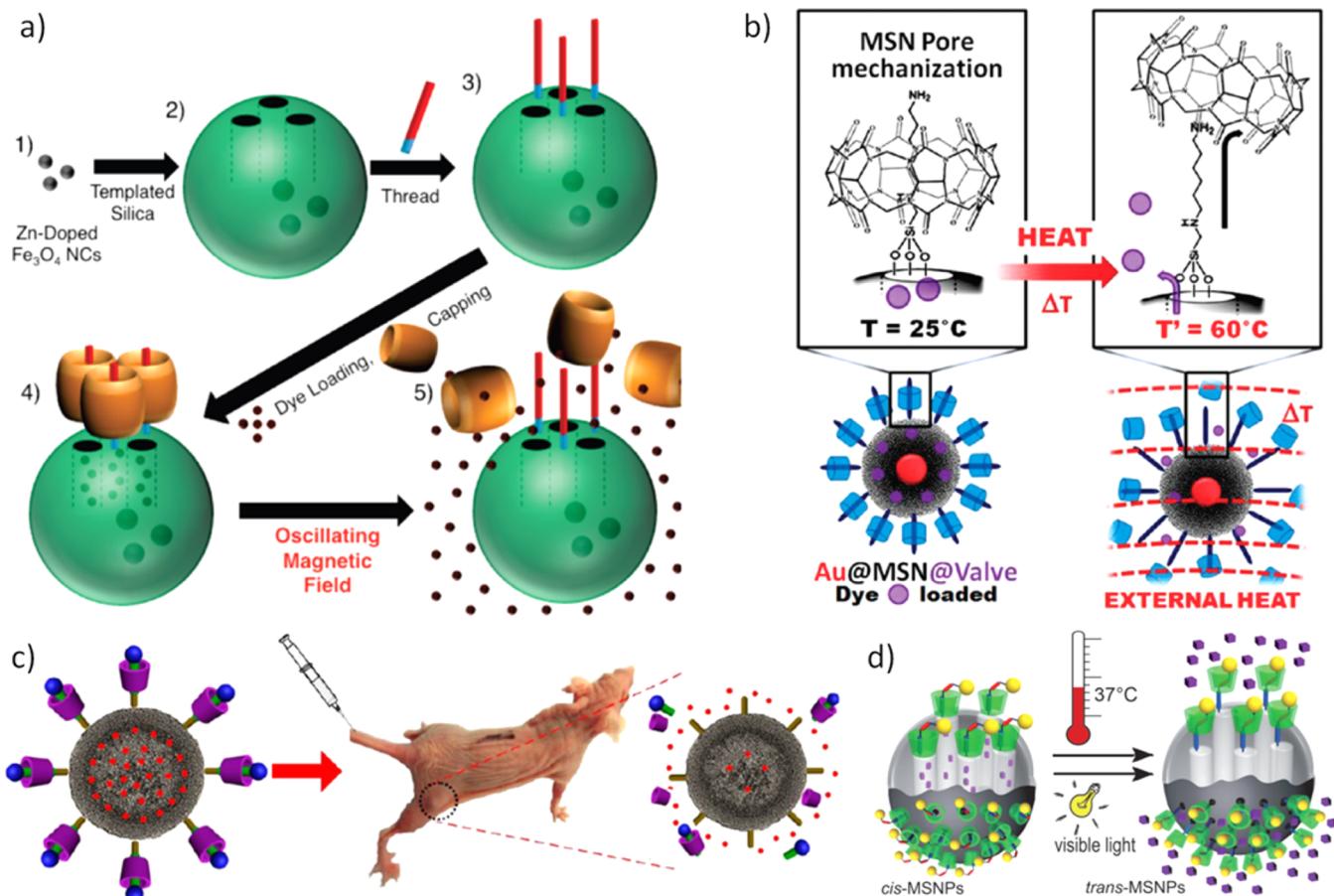


Figure 19. (a) Noninvasive remote-controlled release of drug molecules using magnetic actuation of mechanized nanoparticles. Reprinted with permission from ref 247. Copyright 2013 American Chemical Society. (b) External heating at 60 °C to cause the dissociation of the CB caps from the stalks on Au nanoparticle-encapsulated MSNPs for the cargo release from the mesopores. Reprinted with permission from ref 248. Copyright 2013 American Chemical Society. (c) Hollow nanocontainers functionalized with molecular machines for tumor-targeted therapy in vitro and in vivo. Reprinted with permission from ref 253. Copyright 2013 American Chemical Society. (d) Functional MSNPs for photothermal-controlled drug delivery. Reprinted with permission from ref 254. Copyright 2012 Wiley-VCH Verlag.

(PNIPAm) led to the formation of supramolecular nanoparticles, where the size was tunable by varying the weight ratio of PNIPAm/PEG- β -PCD. A hydrophobic drug, indomethacin, was loaded into the formed supramolecular nanoparticles and can be released in a sustained manner, the rate of which can be controlled by the temperature.²²⁹ Later, they utilized various hydrophobic drugs as the guest molecules to induce the self-assembly of the block copolymer for the nanoparticle formation. The supramolecular nanoparticles showed responsive drug release upon the addition of hydrophobic guest molecules or free β -CD, which could competitively replace the original guest or host moieties, hence triggering the dissociation of the nanoparticles to accelerate the drug release.²³⁰ Although the in vitro and in vivo drug delivery was not conducted for the investigation, they presented a relatively systematic study on the supramolecular nanoparticle formation through the host–guest interaction of block copolymers for controlled drug release.²³¹

In this aspect, Tseng and co-workers also developed a series of supramolecular nanoparticles based on the host–guest interaction between CD and Ad, which are either conjugated on polymers or dendrimers for various biological applications. In 2009, they reported a facile approach to prepare supramolecular nanoparticles by CD/Ad recognition-induced self-assembly of three different building blocks, including Ad-modified polyamidoamine dendrimer (Ad-PAMAM), β -CD grafted branched

polyethylenimine (CD-PEI), and Ad functionalized PEG (Ad-PEG). The unique feature of this work was tunable size of the self-assembled supramolecular nanoparticles ranging from 30 to 450 nm. The studies on the stability, disassembly, size tunability, and whole-body misdistribution of the prepared nanoparticles with two specific sizes were carried out, revealing the critical role of the supramolecular nanoparticle size for in vivo applications.²³² Upon further addition of CPT grafted PEG (CPT-PEG) into previous three building blocks, anticancer drug CPT containing supramolecular nanoparticles with two different sizes was prepared. By using positron emission tomography (PET) imaging technology, the body distribution and tumor accumulation of the formed nanoparticles were evaluated. They then chose an optimized nanoparticle size for in vivo study, indicating enhanced antitumor effect of the CPT containing supramolecular nanoparticles.²³³

Very recently, the same group combined the supramolecular system with magnetic nanoparticles (MNP) to generate a series of supramolecular magnetic nanoparticles (SMNPs) with varied sizes. Anticancer drug Dox was loaded to produce Dox-encapsulated supramolecular magnetic nanoparticles (Dox-SMNPs), and on-demand drug release was triggered by quickly generated thermal energy when applying an external alternative magnetic field (AMF) (Figure 18).²³⁴ In addition to the drug delivery, the CD/Ad-based supramolecular nanoparticles were

also utilized for other biomedical applications. For instance, by encapsulating gold nanoparticles with 2 nm size into the supramolecular nanoparticles, enhanced photothermal therapeutic efficacy against cancer cells was achieved.²³⁵ Another major focus was to use the supramolecular nanoparticles for gene delivery, which will be addressed in detail later.

2.1.2.4. Host–Guest Nanohybrids. **2.1.2.4.1. “On–Off” Switch on Nanoparticles.** The reversibility of host–guest inclusion has paved a new pathway for the design of controlled drug delivery systems by integrating the supramolecular systems with inorganic nanoparticles such as mesoporous silica nanoparticles (MSNPs). MSNPs possess several advantages for drug delivery application, including high specific surface area, large pore volume, uniform pore size, ease of functionalization, and good biocompatibility.^{236–238} A series of controlled drug/gene delivery systems based on MSNPs were developed, and the loaded drugs can be released triggered by external stimuli such as pH change,^{67,239,240} temperature change,²⁴¹ and redox.^{242,243} The fabrication of “nano-gate” on the MSNP surface by utilizing the host–guest complexation to control the cargo loading and release in the mesopores has become a very interesting research topic during the past few years.^{244–246}

Several novel drug delivery systems based on MSNP using the host–guest complex as “nano-gate” were fabricated with responsiveness to external stimuli for remote controlled and noninvasive drug delivery. For example, by combining magnetic nanocrystals with MSNPs where the MSNP surface was modified with pseudorotaxanes as the “nano-gate”, a thermal responsive drug delivery system was achieved (Figure 19a). Upon the application of external magnetic field, the heat generated by the magnetic nanocrystals could effectively destroy the host–guest inclusion of the pseudorotaxanes, resulting in the opening of the mesopores for the drug release.²⁴⁷ In a similar strategy, by embedding gold nanoparticles as the heat generating cores within MSNPs, a mechanized “nano-gate” on MSNPs by the host–guest interaction between CB[6] and *N*-(6-aminoethyl)-aminomethyltriethoxysilane can be opened upon external laser light-induced thermal generation, leading to controlled drug delivery (Figure 19b).²⁴⁸ Furthermore, the Zink group developed a “snap-top” stopper by the host–guest interaction between β -CD and a coumarin derivative covalently linked on the MSNP surface, where the covalent bond between coumarin and MSNPs was cleavable upon UV or two-photon-NIR light irradiation.²⁴⁹ More sophisticated drug delivery systems were also fabricated for dual-responsive and selective cargo release. By anchoring the β -CD ring at the orifice of the mesopores via the disulfide bond linkage, a large size cargo, Hoechst 33342, could be effectively loaded. Meanwhile, a guest molecule methyl orange (MO) was plugged into the cavity of CD ring, blocking a small size cargo, *p*-coumaric acid, within the mesopores. Under acidic condition, unplugging of protonated MO led to the *p*-coumaric acid release, while redox triggered disulfide cleavage caused the removal of the β -CD ring for Hoechst 33342 release.²⁵⁰ Other types of capping molecules, such as pillar[5]arene, have been utilized to fabricate similar mechanized MSNPs for controlled cargo release by pH variation or competitive guest binding.²⁴⁴

In addition to traditional stimulus methods, enzyme-responsive systems could serve as target-specific drug carriers on account of the specificity of the enzyme reaction. For example, Yang and co-workers recently developed a multiresponsive nanovalve, where a CA ring was capped onto choline-containing stalk functionalized MSNPs via the host–guest complexation. The controlled drug release could be triggered not only by

enzyme, but also by pH change and competitive binding.²⁵¹ The same group also employed CB[7] to complex with the azobenzene stalk on MSNPs for light-controlled drug loading and release.²⁵² Liu and co-workers threaded CB[7] onto protonated 1,4-butanediamine stalk functionalized at the orifice of MSNPs. The mesopores were blocked tightly by the host–guest complex between CB[7] and 1,4-butanediamine. Enzymatic products, like lysine and cadaverine, could replace the 1,4-butanediamine unit to form new host–guest complex with CB[7], leading to the activation of the nanovalves for the drug release from the mesopores.²⁴⁵

Aiming at better applicability in the biomedical field, a series of controlled drug delivery systems using CDs as the capping agents were fabricated. The controlled release property of some systems was investigated both in vitro and in vivo. Taking advantage of photothermal-responsive trans–cis isomerization of azobenzene, the movement of α -CD threaded on the surface of MSNPs could be controlled, realizing on-demand drug release from the mesopores. The remote-controlled drug release of the delivery system was carried out in vivo using zebrafish models, showing effective heart failure therapy as compared to free drug (Figure 19d).²⁵⁴ Later, the β -CD/Ad complex on MSNPs was utilized for controlled anticancer drug delivery. The targeting ligand, folic acid, and biocompatible polymer, PEG, were both incorporated onto MSNPs by the CD/Ad host–guest interaction to fabricate multifunctional MSNPs. In vivo study of the delivery system was performed in vivo, indicating significant tumor inhibition capability.^{255,256} More recently, Zhao and co-workers engineered α -CD-based rotaxane onto hollow MSNPs, where folic acid served as not only the targeting ligand, but also the terminal group of the thread. The embedded disulfide bond could fulfill redox-controlled Dox release for cancer therapy in vitro and in vivo (Figure 19c).²⁵³ A similar strategy was recently reported by Porta and co-workers. Folic acid terminated stalk moiety was modified on MSNPs, and the β -CD ring threaded onto the stalk to cap the mesopores. Upon the addition of bioactive molecules, like porcine liver esterase, the functional stalk could be cleaved, leading to controlled drug release. The bioactive nanogated MSNPs were further studied in vitro by delivering anticancer drug CPT into U2OS cells for controlled therapy.²⁵⁷

2.1.2.4.2. Other Nanohybrids with Host–Guest Interaction. Some functional nanomaterials including QDs, Au nanoparticles, magnetic nanoparticles, and graphene oxide have been widely explored for their applications in biomedicine. Rational combination of supramolecular host–guest systems with these nanomaterials has produced many types of multifunctional hybrids, which can serve as novel theranostic platforms in biomedicine field. For instance, Mulder and co-workers reported polymer–lipid nanoparticles constructed on gold nanocrystals. The inclusion of anticancer drug Dox into the cavity of β -CD on the hybrid was utilized for the drug delivery.²⁵⁸ In another case, the host–guest complex between β -CD and anticancer drug CPT was anchored on the surface of magnetic nanoparticle via redox-responsive disulfide bond linkage. The developed drug delivery system was responsive to both reducing agent and external magnetic field. In addition to the controlled drug delivery capacity, this system also allows for in vivo application by magnetic resonance imaging technique.²⁵⁹ The integration of different biological functions into one system provides a new approach to fabricate multifunctional nanosystems. Jung and co-workers developed a multifunctional theranostic system through in situ assembly driven by the host–guest interaction between CB[6] and various functional moieties (Figure 20). Hyaluronic

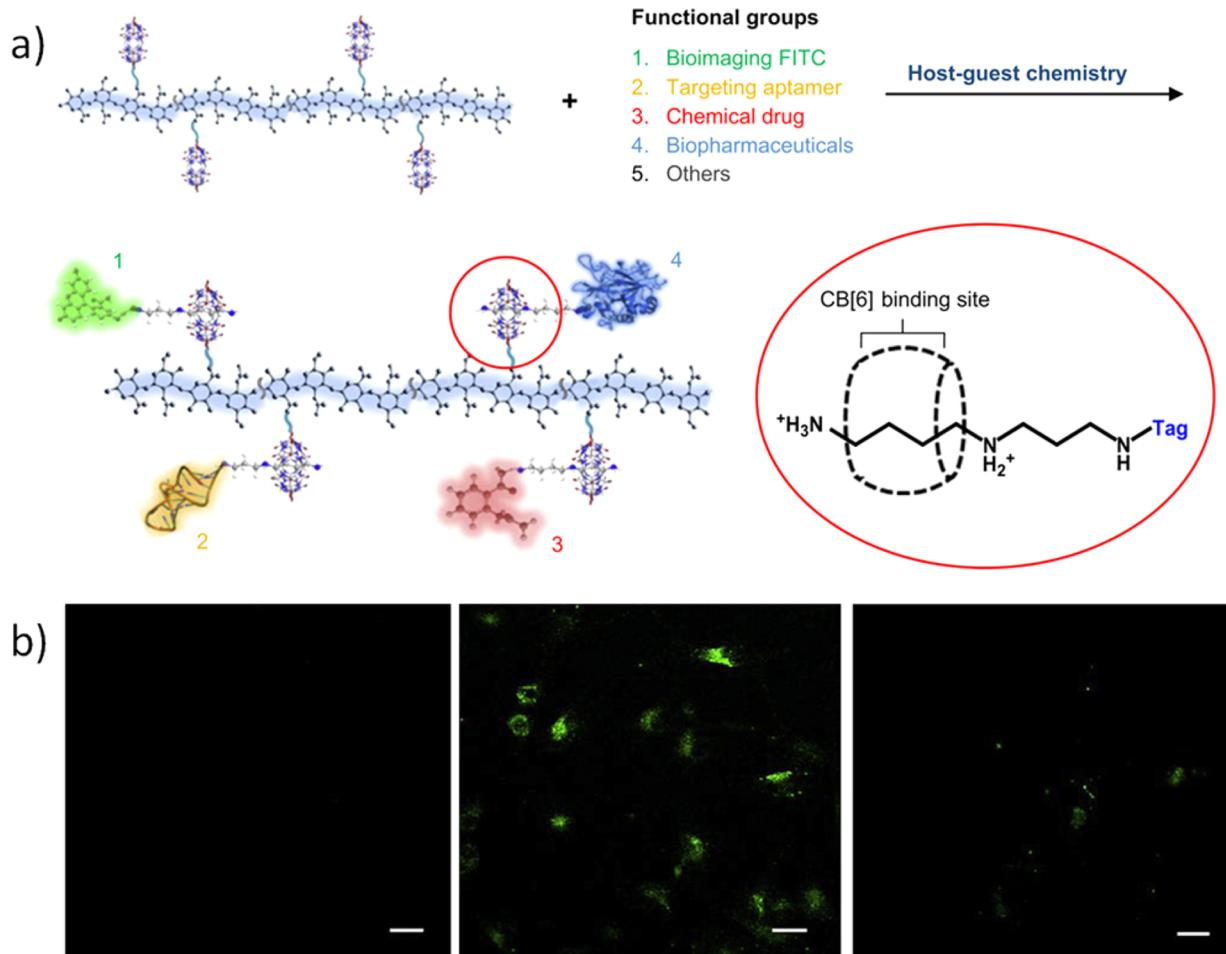


Figure 20. (a) On-demand multifunctional theranostic system constructed by the host–guest interaction between CB[6]-HA and various functional group tagged spermidine. (b) Confocal laser scanning microscopic images of B16F1 cells after incubation with (from left to right) FITC-spmd, FITC-spmd@CB[6]-HA, and FITC-spmd@CB[6]-HA in the presence of 1000-fold molar excess HA at 37 °C for 24 h, respectively. Scale bar = 20 mm. Reprinted with permission from ref 260. Copyright 2011 Elsevier.

acid (HA) was chosen as the polymer backbone on which CB[6] was conjugated. Different functional moieties, such as FITC for bioimaging, anticancer drugs, and targeting aptamer, were linked with spermidine, which can effectively form the host–guest complex with CB[6] in aqueous solution. The work presents a facile method to fabricate functional theranostic nanosystems for the cargo delivery in on-demand manner.²⁶⁰

Although supramolecular system-based drug delivery carriers are beneficial from the advantage of reversibility, practical application inside living organism is still facing considerable difficulties due to the complexity of intracellular conditions. A lot of research has been carried out to address the issue. For instance, Rotello and co-workers reported a supramolecular nanohybrid, where the host–guest complex was anchored on ultrasmall gold nanoparticles. The surface of gold nanoparticles was modified with diaminohexane (AuNP-NH₂), which could thread with CB[7]. The introduction of CB[7] on AuNP-NH₂ greatly reduced the cytotoxicity. Upon the addition of 1-adamantylamine to replace diaminohexane and then result in the uncapping of CB[7] inside the living cells, endosomal escape and *in situ* cytotoxicity of AuNP-NH₂ was activated. This report demonstrated the activation of supramolecular host–guest complex inside living cells, providing a new strategy for the development of theranostic platforms.²⁶¹

2.1.3. Supramolecular Hydrogels. Hydrogels are three-dimensional cross-linked networks, which can hold a large amount of water via surface tension or capillary effect.²⁶² Synthetic polymeric hydrogels were first introduced in late 1950s,²⁶³ and ever since hydrogels have been extensively explored in a broad range of applications. Especially, because of their good biocompatibility and mechanical property similar to that of human tissues, hydrogels have exhibited great potentials for biomedical applications, such as serving as the carriers for the delivery of small chemotherapeutic reagents or biomolecules as well as the scaffolds for artificial tissue construction.^{80,264–267} The preparation methods of hydrogels can be sorted by the driving forces of cross-linking. Covalent chemical bond connections usually afford relatively brittle hydrogels with poor transparency. Such kind of gels cannot be recovered once the cross-linking connection is broken.²⁶⁸ On the other hand, physical connections by noncovalent interactions, such as hydrogen-bonding interaction, electrostatic interaction, and metal–ligand complexation, can yield reversible and versatile hydrogels.²⁶⁹ In particular, the fabrication of hydrogels via the host–guest interaction has attracted significant attention, as the reversibility of host–guest complexation provides the possibility of producing stimuli-responsive and self-healing hydrogels.²⁷⁰

Usually, supramolecular hydrogels formed by the host–guest inclusion can be easily classified into two major groups. The first

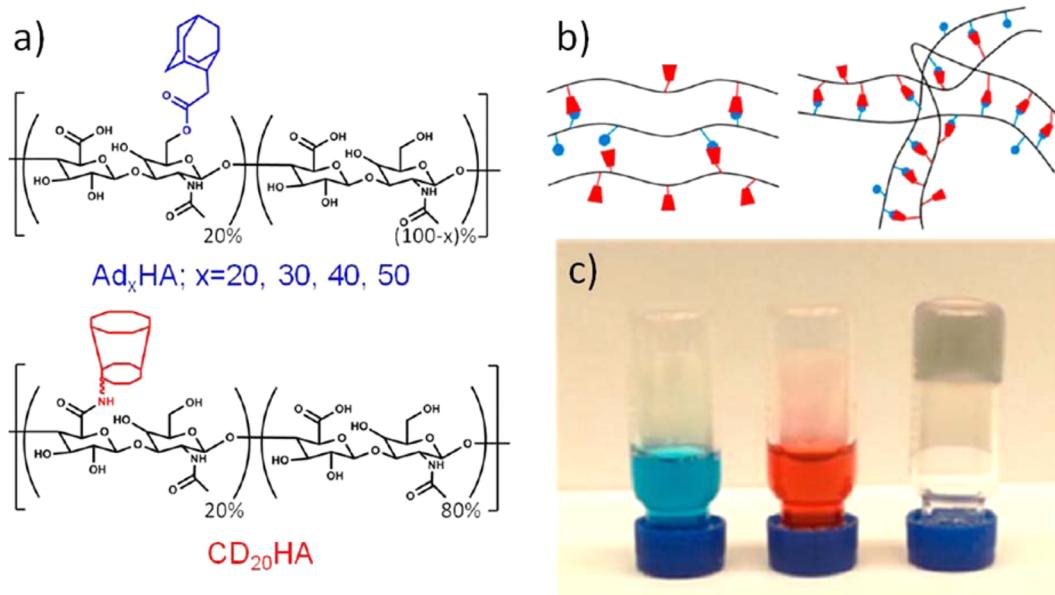


Figure 21. (a) Chemical structure of the host–guest components. (b) Schematic representation of weak network junction (left) and multifold junction (right). (c) Qualitative inversion tests: Ad20HA 5 wt % (blue, left), CD20HA 5 wt % (red, middle), and CD20HA with Ad20HA 5 wt % (purple, right). Reprinted with permission from ref 275. Copyright 2013 American Chemical Society.

type is based on poly(pseudo)rotaxanes by threading macrocyclic molecules onto polymer chains. Hydrogen-bonding interaction between the macrocyclic molecules is the major driving force for the polymer cross-linking. Such kind of supramolecular hydrogel was first reported by Harada and co-workers in 1994, and they carried out a lot of research in this area.^{271,272} Several similar hydrogels were reported in early years.^{273,274} For the second type, macrocyclic host molecules and guest molecules are both conjugated onto polymers as the side chains. The host–guest complexation between the host and guest can directly induce the cross-linking formation between two types of polymer conjugates (Figure 21).^{275–278} In some cases, only guest molecules were conjugated onto the polymers, and macrocyclic host molecules were added to form 1:2 complex with the guest moieties on the polymers to make the hydrogels.^{279–281}

In addition to providing the cross-linking connections by the host–guest complexation, introducing macrocyclic molecules into hydrogels can alter the hydrophilic property of the hydrogels to enhance their biocompatibility. As for using hydrogels for the drug delivery, another important purpose of introducing macrocyclic molecules is to encapsulate various chemotherapeutic reagents via the host–guest inclusion or hydrophilic interactions.²⁸² In this case, a common strategy for the drug delivery is to entrap chemotherapeutic reagents or therapeutic biomolecules inside the hydrogel matrix. The drugs or biomolecules then can be released from hydrogels in a sustained manner either by free diffusion or by biodegradation of hydrogels.⁸¹ The affinity between delivered cargos and the polymer matrix can greatly influence the release rate and release profile. Nielsen and co-workers prepared a hydrogel by using poly vinylpyrrolidone/poly ethylene glycol-dimethacrylate (PVP/PEG-DMA), and CDs were covalently conjugated onto the polymer chain to slow the release rate of hydrophilic model drug, ibuprofenate (IBU).²⁸³ In another study, researchers loaded lysozyme, β-estradiol, and quinine into CD-PEG-based hydrogels. The results indicated that the release rates of these model drugs were closely dependent on their interaction with

hexamethylated β-CD inside the hydrogels.²⁸⁴ Hennink and co-workers reported a supramolecular hydrogel consisting of β-CD and cholesterol-bearing PEG. The degradation of the hydrogel could cause a quantitative release of entrapped proteins, presenting a promising protein delivery system.²⁸⁵ Scherman and co-workers developed a supramolecular hydrogel by using CB[7] to connect polymer matrix, which showed extremely high water content (up to 99.5%). The loaded protein could be released from the hydrogel in a sustained manner.²⁸¹

By taking advantage of the reversibility of host–guest complex, stimuli-responsive hydrogels were developed. For instance, Stoddart and co-workers reported a light-responsive hydrogel system based on the complexation between azobenzene and β-CD.²⁸⁶ Similar light-responsive hydrogels were reported by Harada and co-workers as well.²⁸⁷ pH-responsive^{288,289} and redox-responsive²⁹⁰ hydrogels were also reported. Moreover, Scherman and co-workers developed a multiresponsive supramolecular hydrogel by using CB[8] to connect guest moiety-bearing poly(vinyl alcohol). The hydrogel was responsive to the temperature change, chemical potential, and competing guests.²⁹¹ An interesting application of such kind of hydrogels is controlled drug delivery. Specifically, Chen et al. reported a hydrogel system via the host–guest interaction.²⁹² A “glue molecule”, riptycenederived macrotricyclic polyether (TDMPE), was utilized to connect the guest moiety, dibenzylammonium (DBA), covalently conjugated onto the copolymer chains. The formed supramolecular hydrogel showed reversible sol–gel transition upon pH or thermal stimulus. In addition, the authors encapsulated squaraine dyes inside the polymer network and demonstrated controlled release property. Zhang and co-workers reported a similar strategy to prepare bioactive supramolecular hydrogel by using α-CD as the host molecule to form a three-dimensional polymer network.²⁹³ In this case, a short polymer side chain, poly(ethylene glycol) methyl ether, was conjugated onto the heparin polymer main chain. The α-CD ring was threaded onto multiple PEG side chains to connect the polymers and form cross-linked polymer network. The formed supramolecular hydrogel can encapsulate a

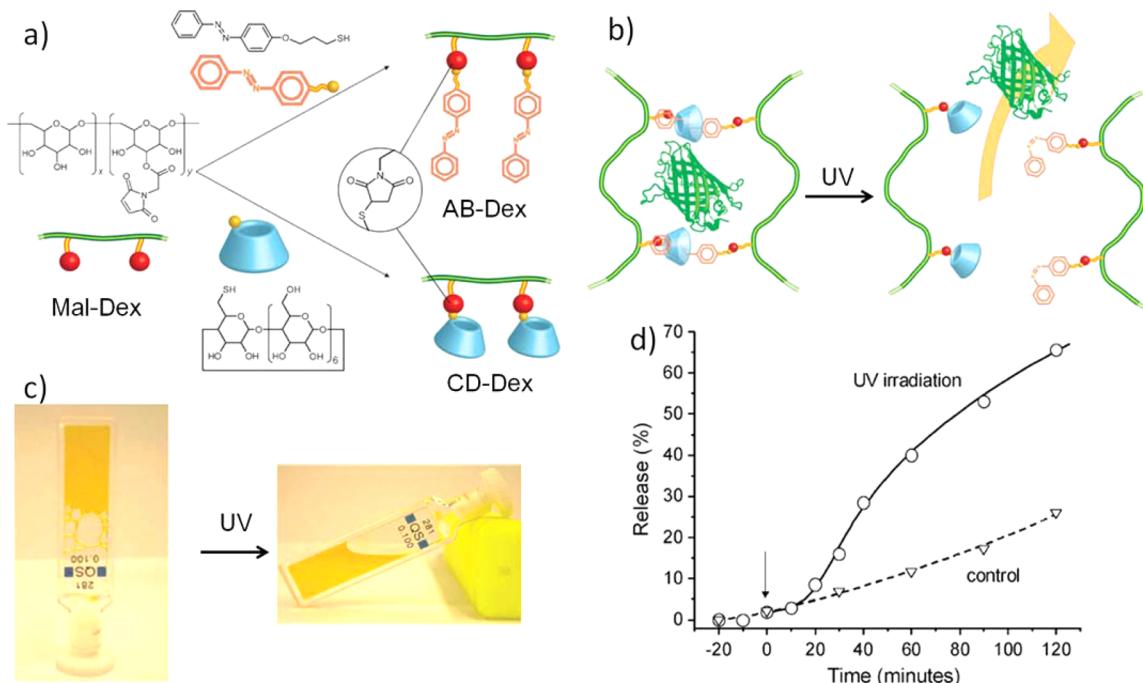


Figure 22. (a) Preparation of azobenzene-modified dextran (AB-Dex) and cyclodextrin-modified dextran (CD-Dex) through the thiol-maleimide reaction. (b) Photoresponsive protein release from a hydrogel composed of *trans* AB-Dex and CD-Dex. Upon the UV light irradiation, the azobenzene moiety isomerizes from *trans* to *cis* configuration, resulting in the dissociation of cross-linked hydrogel to release entrapped protein into the media. (c) Photographs of a mixture of AB-Dex (67 mg mL^{-1}) and CD-Dex (100 mg mL^{-1}) in PBS before (left) and after UV light irradiation (right). (d) Release of green fluorescent protein (GFP) from the supramolecular hydrogel. From the time 0, the sample was placed under UV light. With a delay of 10 min, GFP was released from the hydrogel matrix. Reprinted with permission from ref 295. Copyright 2010 The Royal Society of Chemistry.

protein model drug, bovine serum albumin, and demonstrated tunable release properties upon different amounts of α -CD or heparin-PEG conjugate used. They used α -CD and a polymeric prodrug, PEGylated indomethacin (MPEG-indo), to prepare another supramolecular hydrogel and studied its properties, including the gelation kinetics, mechanical strength, shear-thinning behavior, and thixotropic response. A sustained release of loaded protein from the hydrogel was also demonstrated.²⁹⁴

The new hydrogel was further evaluated *in vitro* with Hep-2 cell line to test its feasibility as an injectable drug delivery system. The results indicated that the encapsulated prodrug, MPEG-indo, could be released sustainably, while the hydrogel delivery system could still maintain its bioactivity. Peng and co-workers fabricated a light-responsive hydrogel (Figure 22) via the host–guest interaction between β -CD and azobenzene for protein delivery.²⁹⁵ They separately functionalized dextrans with azobenzene and β -CD through thiol-maleimide coupling. When mixing two types of dextran polymers, the inclusion of *trans* azobenzene with β -CD would induce the gel formation. Upon light irradiation at 365 nm, the *trans*-to-*cis* isomerization of azobenzene led to the dissociation of the β -CD/azobenzene complex, resulting in the dissolution of the hydrogel. Simultaneously, the pre-encapsulated protein inside the hydrogel was released.

A lot of drug delivery systems based on host–guest interactions have been developed over the past decades. In addition to the extensive studies with biocompatible CDs, new research work using CAs and CBs has also been explored for drug delivery applications. Simple host–guest complexation between drugs and macrocyclic molecules provides an easy strategy to achieve improved drug delivery in terms of enhanced drug solubility, high bioavailability, and tunable degradation of drugs

in biological environment. A variety of nanosized supramolecular systems, including vesicles, micelles, and nanoparticles, have greatly enriched the pool of biomedical tools for drug delivery. However, host–guest interaction-based systems have never been confined within drug delivery, and their applications cover a wide range in biomedical field. Other applications including gene delivery, bioimaging, and photodynamic therapy will be discussed.

2.2. Gene Delivery

Gene therapy has attracted increasing interest since its discovery nearly 30 years ago. A successful gene delivery is strongly dependent on the gene carriers. The application of traditional viral delivery has been challenged by its toxicity issue, immunogenicity, and low scaled-up capability. Therefore, there has been long a scientific demand for developing nonviral gene delivery systems that can overcome these drawbacks.²⁹⁶ However, nonviral gene delivery method is still facing difficulties brought by natural barriers, including blood circulation, cell membrane, and nucleus membrane. Moreover, the biodegradation of nucleic acid and the escape of delivered gene inside target cells or cell nucleus are also critical factors under consideration for nonviral gene delivery. The nonviral gene delivery has been advanced by the fast development of nanotechnology, leading to numerous novel gene delivery systems based on functional nanomaterials, such as various nanoparticles, polymers, and graphene oxide.

The application of supramolecular chemistry for gene delivery has been a hot research topic in biomedical field.^{297,298} Simple cationic macrocyclic molecules, like star-shaped derivatives or amphiphilic macrocyclic molecules, were utilized to condense nucleic acid by electrostatic interaction.^{96,97,299–306} Cell-penetrating peptide conjugated macrocyclic molecules were

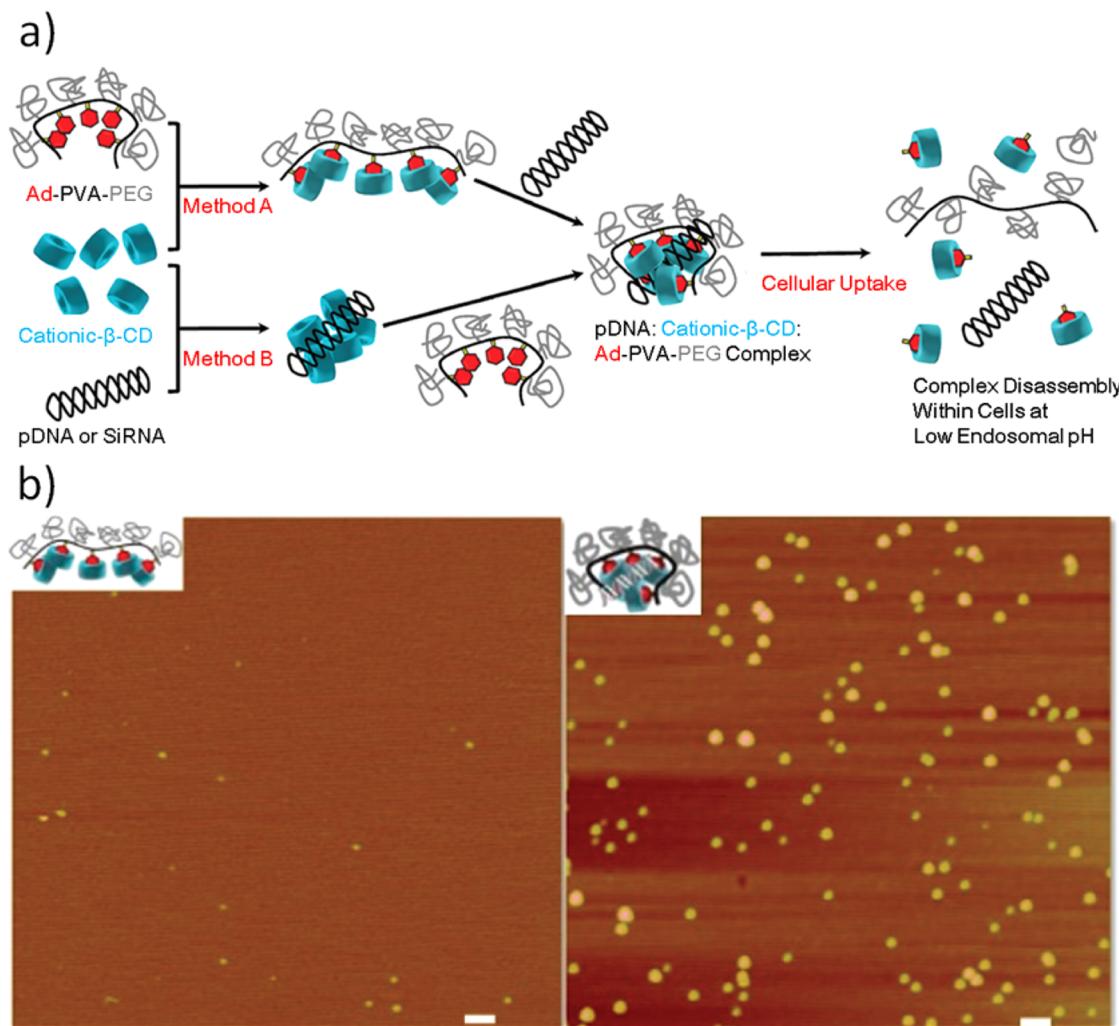


Figure 23. (a) pDNA delivery system prepared based on noncovalent assembly of bioresponsive amino- β -CD/Ad-PVA-PEG complex. (b) AFM images of (left) Chol-PVA-PEG:3 and (right) Chol-PVA-PEG:3:siRNA at N/P = 10. Scale bar = 100 nm. Reprinted with permission from refs 316 and 317. Copyright 2012 American Chemical Society.

fabricated to enhance the cellular uptake and DNA delivery efficacy.⁹³ Researchers also chemically conjugated macrocyclic molecules onto cationic polymer chains to enhance the gene delivery efficacy, while decreasing the toxicity of the cationic polymers.^{307,308} Supramolecular micelles constructed by amphiphilic macrocyclic molecules were used to encapsulate genes by supramolecular self-assembly for accomplishing the gene delivery.^{291,309–313} In addition, targeting ligand folic acid was conjugated onto such micelles to realize targeted gene delivery.³¹⁰ In particular, CDs and their derivatives have been widely utilized in constructing supramolecular gene delivery systems, mainly due to their superior biocompatibility.^{78,79,314} Meanwhile, there were relatively less reports about CA- and CB-based gene delivery systems. Considering a large amount of scientific studies about the application of supramolecular chemistry in gene delivery, supramolecular nonviral gene delivery carriers based on the host–guest interaction will be discussed in this section.

2.2.1. Supramolecular Nanoparticles for Gene Delivery. The construction of gene encapsulated supramolecular nanoparticles via the host–guest interaction is a popular strategy to create nonviral gene delivery systems. Davis and co-workers developed promising supramolecular nanoparticles for ther-

apeutic siRNA delivery.³¹⁵ Actually, it was for the first time using supramolecular nanoparticles for cancer targeted siRNA delivery in clinical application. The delivery carrier consists of four parts, including CD-based linear polymer, Ad terminated hydrophilic polymer PEG (PEG-Ad), a human transferring (hTf) protein targeting ligand containing PEG-Ad (Ad-PEG-hTf), and siRNA duplex. The nanoparticles were formed by noncovalent self-assembly driven by the host–guest interaction between CD and Ad. The targeting ligand hTf was modified on the nanoparticle surface through the CD/Ad complexation as well. The obtained results show obvious reduction of specific mRNA and target protein level as compared to predosing tissue, indicating good efficacy of the *in vivo* siRNA delivery system. Similar supramolecular nanoparticle-based gene delivery system was also reported by Thompson and co-workers, in which the poly(vinyl alcohol) (PVA) polymer main chain bearing PEG was modified with cholesterol pendant via acid-sensitive benzylidene acetal linkage (Figure 23).³¹⁶ By utilizing the host–guest interaction between cationic β -CD derivative and cholesterol unit, the supramolecular nanoparticles were formed. siRNA could be encapsulated into the supramolecular nanoparticles before or after the self-assembly process. Green fluorescence protein (GFP) expressed cell lines were used for the *in vitro* siRNA

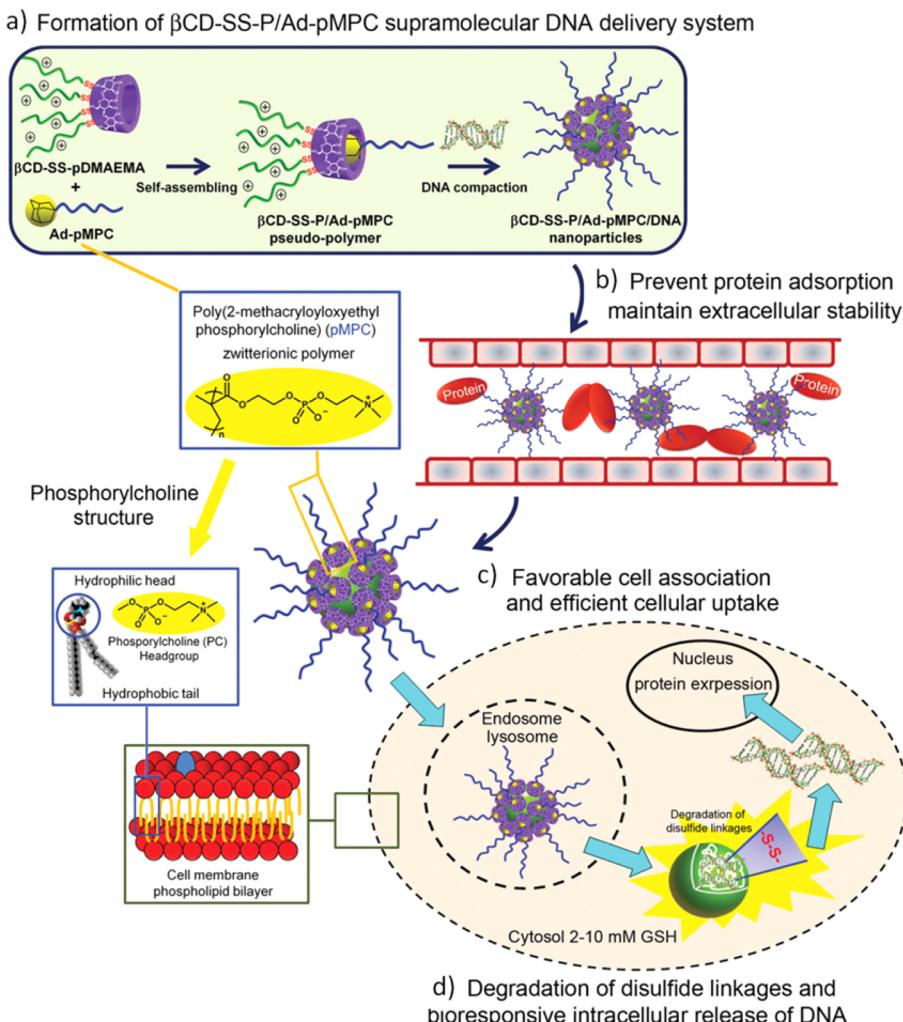


Figure 24. Conceptual illustration of β -CD-SS-P/Ad-pMPC supramolecular gene delivery system. (a) Formation of β -CD-SS-P/Ad-pMPC pseudodiblock copolymer via the host–guest interaction, followed by the DNA compaction to form β -CD-SS-P/Ad-pMPC/DNA polyplex. (b) Protein-repellent properties of pMPC imparting the extracellular stability of the polyplex. (c) Favorable cellular association and efficient cellular uptake on account of the zwitterionic phosphocholine structure of pMPC corona. (d) Rapid intracellular unpacking and release of DNA due to redox-sensitive disulfide linkage, followed by efficient gene transfection. Reprinted with permission from ref 324. Copyright 2014 Wiley-VCH Verlag.

delivery, and anti-GFP siRNA was utilized for the gene silence study. After the internalization into target cells, the cleavage of acid-sensitive linkage led to the disassociation of the supramolecular nanoparticles and thus the release of encapsulated siRNA. Moreover, the entire complex could act as a low-toxic nonviral gene delivery system showing effective gene silence capability when compared to traditional transfection reagent, like PEI or Lipofectamine 2000. This novel gene delivery system was also expanded for plasmid DNA (pDNA) delivery (Figure 23).³¹⁷ In vitro study using HeLa cell line showed very low toxicity (10³-fold lower) of the supramolecular nanoparticles, and superior gene transfection efficacy by delivering mhGFP pDNA into the cells.

As mentioned earlier, Tseng and co-workers made considerable contributions in developing novel supramolecular nanoparticles for theranostic applications, especially for antitumor drug delivery and simultaneous bioimaging. They also expanded the developed supramolecular nanoparticles for gene delivery.³¹⁸ Similar components, including cationic Ad-PAMAM, cationic CD-PEI, and Ad-modified (Ad-PEG), were utilized for the supramolecular assembly via the host–guest interaction between β -CD and Ad unit. A series of nanoparticles with various sizes

ranging from 30 to 450 nm were prepared. It was anticipated that anionic pDNA was encapsulated into the supramolecular nanoparticles by electrostatic interaction with cationic PAMAM or PEI used in the nanoparticle preparation. They then combined this supramolecular assembly method with a self-designed digital microreactor to develop a rapid pathway for screening diverse DNA-encapsulated supramolecular nanoparticles.³¹⁹ By such systematical alternation of several parameters, including the nanoparticle size and surface chemistry, the optimized DNA-encapsulated supramolecular nanoparticles were identified.

A series of gene delivery systems based on the host–guest complexation of CD derivatives with Ad-containing guests were reported by Li and co-workers. They initially prepared 2-hydroxypropyl- γ -CD cross-linked low molecular weight PEI. Targeting peptide MC-10 was covalently conjugated onto the functionalized PEI via the disulfide linkage. The obtained polymer could condense DNA to form nanoparticles about 170–200 nm in diameter. Successful in vitro and in vivo gene delivery toward target cells and tumor on mouse models was achieved, and enhanced antitumor effect was demonstrated.³²⁰ They also prepared a star-shaped polymer with cationic γ -CD,

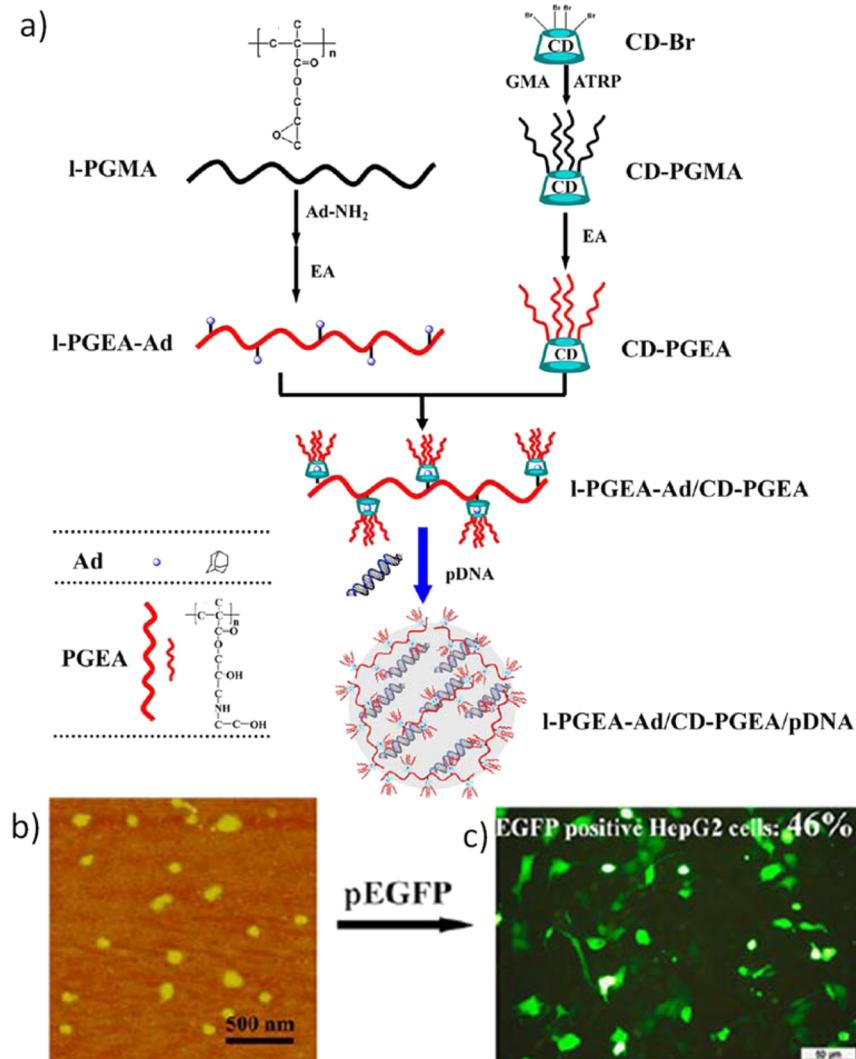


Figure 25. (a) Schematic illustration for the preparation of I-PGEA-Ad/CD-PGEA/pDNA complex. (b) AFM image of I-PGEA-Ad/CD-PGEA/pDNA complex. (c) Representative image of EGFP expression mediated by the I-PGEA-Ad/CD-PGEA/pDNA complex. Reprinted with permission from ref 327. Copyright 2013 American Chemical Society.

which was further connected with folic acid via the disulfide linkage. Targeted gene transfection efficacy was evaluated on both folic acid receptor positive and negative cells, that is, KB cells and A549 cells, respectively.³²¹ In addition, several gene delivery systems based on supramolecular self-assembly were developed by taking advantage of the host–guest inclusion. In 2011, they reported a gene delivery system based on water-soluble chitosan-PEI-β-CD copolymer, which could effectively bind pDNA and siRNA to form spherical nanoparticles via the electrostatic interaction.³²² The presence of β-CD ring allowed for further PEGylation on the nanoparticle surface by facile host–guest complexation with Ad-modified PEG (Ad-PEG). Gene transfection efficacy was investigated using both pDNA encoding luciferase and siRNA against luciferase in several cell lines, including HEK293, COS7, and L929 cells. Afterward, a redox-responsive and FGFR (fibroblast growth factor receptors)-targeted gene delivery system was reported, in which supramolecular host–guest inclusion between β-CD and Ad served as the driving force for the nanoparticle formation.³²³ Specifically, the host segment (MPC) contains β-CD and cross-linked PEI that was conjugated with MC11 peptide, a targeting ligand toward FGFR. The guest segment was disulfide bond

linked Ad-SS-PEG. The host–guest interaction between β-CD and Ad led to the formation of MPC/Ad-SS-PEG polycation, which could condense plasmid DNA to generate supramolecular nanoparticles with sizes from 100 to 200 nm. The redox-responsive disulfide bond could be cleaved after internalization of the nanoparticles into cells, leading to enhanced endosomal escape capability. Tumor targeted gene delivery and transfection were evaluated both *in vitro* and *in vivo* by using tumor-bearing mouse models, indicating promising application for cancer therapy.

More recently, they reported a multifunctional gene delivery system based on supramolecular nanoparticles formed by a similar strategy (Figure 24).³²⁴ The difference was that poly(2-dimethylaminoethyl methacrylate) (pDMAEMA) was used as the polycation to replace PEI in the previous case and conjugated with β-CD via the disulfide bond linkage (β-CD-SS-pDMAEMA). Poly(2-methacryloyloxyethyl phosphorylcholine) (pMPC) was used to replace PEG and conjugated with Ad. By introducing zwitterionic phosphorylcholine into the nanoparticles, the obtained gene delivery system could effectively prevent the protein adsorption and thus improve the serum tolerance. Another work also reported such biocleavable

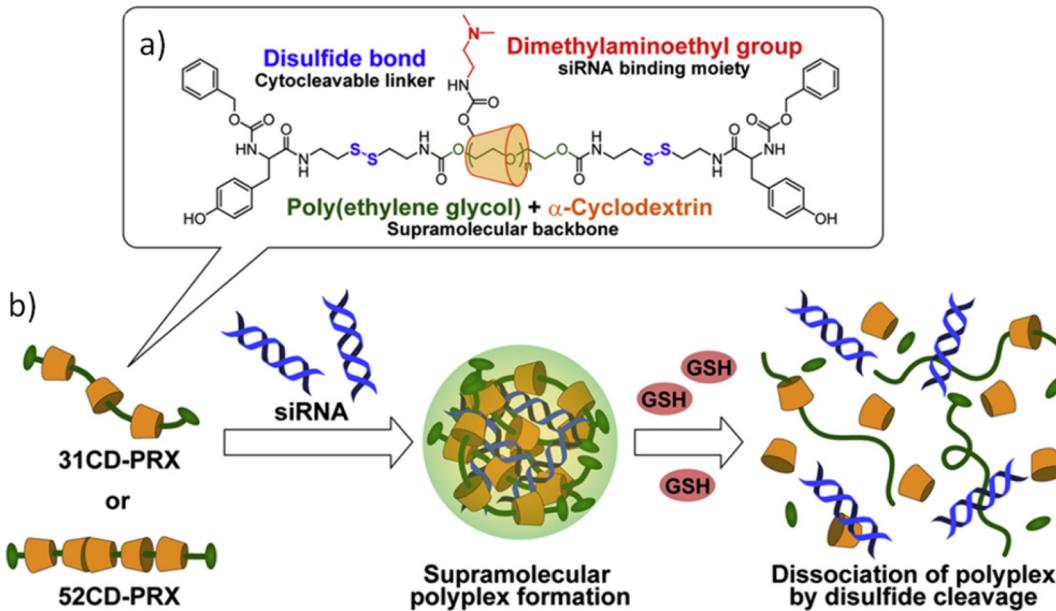


Figure 26. (a) Chemical structure of cyto-cleavable DMAE-PRX; (b) supramolecular polyplex formation between siRNA and DMAE-PRX and the cytoplasmic release of siRNA through the cleavage of terminal disulfide linkage by intracellular glutathione (GSH). Reprinted with permission from ref 328. Copyright 2013 Elsevier.

pseudoblock copolymers-based responsive gene delivery carriers.³²⁵ The supramolecular gene delivery system consists of bioreducible CD-SS-pDMAEMA as the host and Ad linked poly(poly(ethylene glycol)ethyl ether methacrylate) (Ad-pPEGEEEMA) as the guest. The noncovalent interaction between CD and Ad allowed for facile preparation of the polymer/pDNA complex by entrapping pDNA inside the pDMAEMA chains. Detailed investigations were carried out to study the DNA binding capability, in vitro cytotoxicity, gene transfection efficiency, as well as antitumor efficacy. This gene delivery system combined several advantages of bioreducible disulfide bond-containing polycation as well as biocompatible CD and pPEG.

Xu and co-workers employed a different method to prepare supramolecular nanoparticles via the host–guest self-assembly for the purpose of gene delivery. They synthesized a series of star-like gene carriers using β -CD as the core that was covalently conjugated with piperazine (PP)-, *N*-(aminoethyl)piperazine (AEPP)-, or *N*-(3-aminopropyl)-2-pyrrolidinone (APP)-functionalized poly(glycidyl methacrylate), denoted as CD-PGPP, CD-PGAEPP, and CD-PGAPP, respectively. The gene delivery carriers showed lower toxicity and better gene transfection performance in comparison to PEI (25 kDa).³²⁶ On the basis of this work, they further prepared β -CD-containing ethanolamine-functionalized poly(glycidyl methacrylate) (CD-PGEA) star polymer, and also Ad-modified linear polymer PGEA (l-PGEA-Ad). By the host–guest inclusion between β -CD and Ad, they successfully produced a pseudocomb polycation, which could condense plasmid DNA to form supramolecular nanoparticles, the l-PGEA-Ad/CD-PGEA/pDNA complex (Figure 25). The gene transfection efficacy by the supramolecular nanoparticles was higher than the case only using CD-PGEA.³²⁷

2.2.2. Rotaxane/Polyrotaxane for Gene Delivery. In addition to the formation of supramolecular nanoparticles by the host–guest complexation, Yui and co-workers applied polyrotaxanes into siRNA delivery to realize therapeutic gene delivery application (Figure 26).³²⁸ Although almost the same strategy

was employed for the siRNA delivery as compared to previous cases for the pDNA delivery, in this study, detailed investigation on the relationship between the chemical structure of the polyrotaxane and its siRNA delivery efficiency was carried out. For example, they revealed that a high content of dimethylaminoethyl (DMAE)- α -CD threaded on the PEG polymer could present better capability in forming the complex with siRNA and also higher intracellular uptake of siRNA. Successful siRNA transfection was achieved by silencing luciferase expression in HeLa cells. In addition, enhanced gene silencing was observed from the polyrotaxane with biocleavable disulfide group as compared to the one without the disulfide group. Harashima and co-workers have developed a nonviral gene delivery vehicle using polyrotaxane.^{329,330} Typically, they first synthesized dimethylaminoethyl-modified α -CD (DMAE- α -CD), which was threaded onto PEG. However, the two terminals of PEG polymer were capped with benzyloxycarbonyl tyrosine through a disulfide bond linkage. The positive charge from the amino group on DMAE- α -CD could interact with negatively charged pDNA to form polyrotaxane/pDNA complex. Upon the internalization of the complex in the target cells, intracellular glutathione (GSH) could cleave the disulfide bond to result in the dissociation of the supramolecular complex, leading to the pDNA release. The results indicate the endosomal escape of pDNA, which was further delivered into the cell nucleus for therapy. Afterward, they optimized the preparation parameters of the supramolecular complex for enhancing the gene transfection efficacy, including the number and spatial density of DMAE- α -CD threaded on the PEG polymer.³³¹

The endosome/lysosome escape of siRNA is a critical step for the siRNA delivery. In previous cases discussed, the biodegradation of the polyrotaxanes was all based on the reduction-responsive disulfide bond, which could be effectively cleaved in the cytoplasm after the endosome escaping. Thus, the chemical structure of the PEG backbone was altered. Instead of using disulfide bond to link the capping moiety at the two terminals of PEG polymer, acid-cleavable 3-sulfanylpropionyl

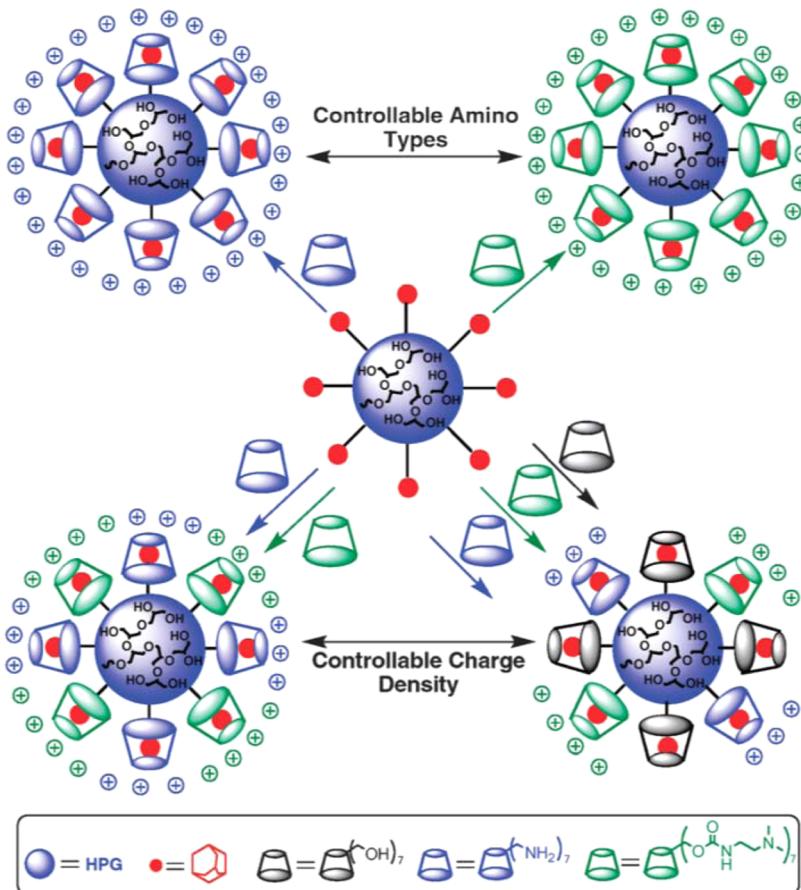


Figure 27. Charge-tunable supramolecular dendritic polymer (SDP) constructed through β -CD/AD host–guest interactions. Reprinted with permission from ref 333. Copyright 2011 Royal Society of Chemistry.

Table 3. Summary of Host–Guest Supramolecular Systems for Gene Delivery

host molecules	guest molecules	gene delivery	ref
β -CD-based linear polymer	Ad terminated hydrophilic polymer PEG	in vivo siRNA delivery against expression of M2 subunit of ribonucleotide reductase (RRM2)	315
monosubstituted cationic β -CD derivatives	poly(vinyl alcohol)(PVA) polymer bearing PEG and cholesterol (Chol): Chol-PVA-PEG	in vitro siRNA delivery against GFP expression	316
amino- β -CD	Ad-PVA-PEG	in vitro delivery of pDNA encoding GFP	317
cationic CD-grafted branched polyethylenimine (CD-PEI)	Ad-grafted polyamidoamine dendrimer (Ad-PAMAM) and Ad-PEG	in vitro delivery of pDNA encoding GFP in U87, 3T3, and MCF-7 cells	318,319
β -CD-cross-linked-PEI conjugated with MC11 peptide targeting FGFRs (MPC)	adamantyl-SS-PEG (Ad-SS-PEG)	in vitro delivery of pDNA encoding luciferase in PC-3, Hep G2, HeLa, and SKOV-3 cells, in vivo tumor targeted delivery of luciferase gene in living mice	323
poly(2-dimethylaminoethyl methacrylate) conjugated with β -CD via disulfide bond (β -CD-SS-pDMAEMA)	poly(2-methacryloyloxyethyl phosphorylcholine) (pMPC) conjugated with Ad (Ad-pMPC)	luciferase and GFP reporter genes, therapeutic p53 anticancer gene was delivered into MCF-7 breast cancer cells	324
β -CD-containing ethanolamine-functionalized poly(glycidyl methacrylate) (CD-PGEA) star polymer	Ad-modified linear polymer PGEA (I-PGEA-Ad)	GFP protein gene transfection in HepG2 and HEK293 cell lines	327
CD-containing star-shaped poly(2-dimethyl amino)ethyl methacrylate (CD-SS-pDMAEMA)	Ad linked poly(poly(ethylene glycol) ethyl ether methacrylate) (Ad-pPEGEEMA)	luciferase gene transfection in HepG2 and COS7 cells	325
(DMAE)- α -CD	PEG polymer	siRNA transfection silencing luciferase expression in HeLa cells	328
(DMAE)- α -CD	PEG terminated with benzoyloxycarbonyl tyrosine via disulfide linkage	pDNA transfection in NIH3T3 cells	329–331
DMAE- α -CD	PEG terminated with N-benzoyloxycarbonyl-L-tyrosine via 3-sulfanylpropionyl ester linkage	in vitro siRNA delivery against luciferase reporter in HeLa cells	332
cationic β -CD derivatives with varied amino groups	Ad-modified hyperbranched polyglycerol (HPG) dendrimer (HPG-Ad)	in vitro pDNA delivery expressing luciferase in COS-7 cells	333

ester linkage was used to connect the stopper unit, *N*-benzoyloxycarbonyl-L-tyrosine.³³² In this case, after the poly-

rotaxane/siRNA complex was endocytosed by cells, the acidic environment of endosome could effectively cleave the 3-

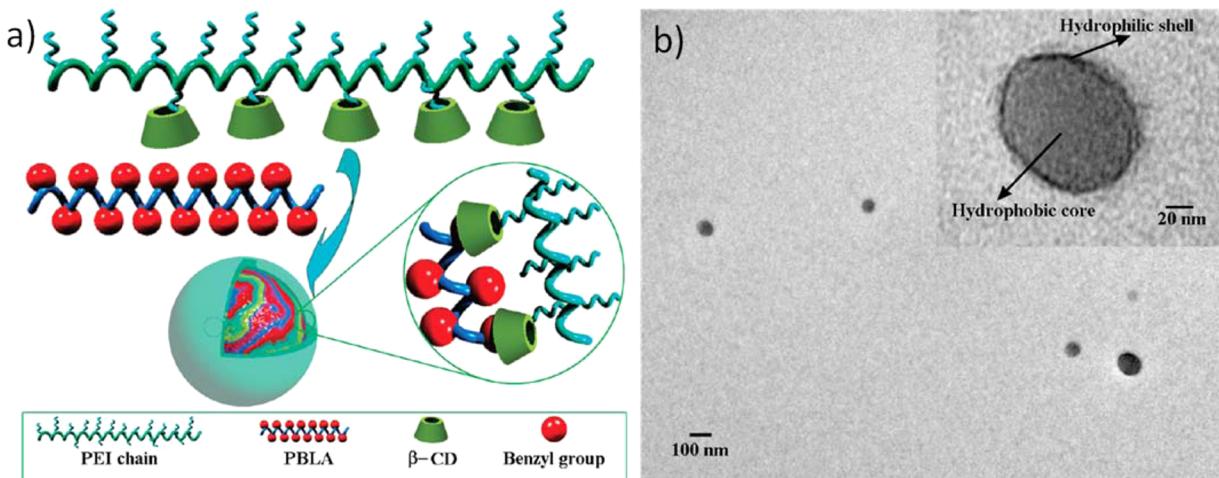


Figure 28. (a) The core–shell assemblies fabricated on the basis of PEI-CD/PBLA through a host–guest interaction. (b) TEM image of PEI-CD/PBLA assemblies after staining with phosphotungstic acid. Reprinted with permission from ref 340. Copyright 2010 American Chemical Society.

sulfanylpropionyl ester linkage, and thus the whole complex was degraded into individual components. The increase of the DMAE- α -CD concentration inside the endosome resulted in selective endosomal membrane destabilization, so that the siRNA release into the cytoplasm enabled the gene silence. The gene silence activity of this novel acid-responsive polyrotaxane was compared to previous reduction-responsive cases, and the enhancement of gene silence capability was observed.

2.2.3. Other Host–Guest Gene Delivery Systems. By taking advantage of facile host–guest complexation, Zhu and co-workers developed a charge-tunable dendritic polycation for the gene delivery (Figure 27).³³³ They functionalized Ad molecule onto hyperbranched polyglycerol (HPG) dendrimer to yield HPG-Ad. Two different cationic β -CD derivatives with varied amino groups were prepared. The host–guest interaction between Ad and β -CD or β -CD derivatives not only stabilizes the supramolecular dendritic polymer (SDP) in aqueous solution, but also provides a facile strategy to tune the surface charge of the SDP by varying the ratio of β -CD derivatives with different amino groups for changing the charge distribution and charge density. The authors used this method to develop a series of SDPs with varied surface charge properties and investigated their gene transfection efficacy in COS-7 cell line by using luciferase assay, through which the gene transfection efficiency of the SDPs was identified. By using bare β -CD to decrease the surface charge density of SDPs, the transfection efficiency of obtained SDPs dramatically decreased. The gene transfection results were further confirmed by using pDNA-encoding GFP as the gene transfection model. The gene transfection efficacy of the optimized SDPs was comparable to that of traditional nonviral gene delivery agent, that is, branched PEI (25 kDa). This research presented an easy method to fabricate a gene delivery vehicle with tunable surface charge and gene transfection efficiency.

As seen from Table 3, the host–guest interaction-based gene delivery systems mainly consist of CDs and their derivatives, which can be explained by their superior biocompatibility and relatively easy modifications. In most cases, charge interaction between cationic moieties of the supramolecular delivery systems and negatively charged genes is the driving force for the gene condensation. Therefore, either cationic CD derivatives or their

host–guest complexes with cationic segments are used for such application. The access to the empty cavity of CD host molecule provides the opportunities for drug encapsulation, which inspired the applications for drug/gene codelivery using single supramolecular systems.

2.3. Drug/Gene Codelivery

Although chemotherapy has been widely utilized for the treatment of many types of cancers, the therapeutic efficacy is sometimes hampered by drug resistance effect when only treated with anticancer drugs.^{334,335} Thus, the codelivery of traditional anticancer drugs and therapeutic genes has been developed to enhance chemotherapy efficacy via synergistic treatment.^{336–338} On one hand, therapeutic genes can be delivered into cancer cells and induce specific protein expression that against cancer growth. On the other hand, RNA interfering (RNAi) technique could enhance the sensitivity of cancer cells to anticancer drugs by selectively silencing certain functional protein expression, like antiapoptotic protein Bcl-2. RNAi is usually achieved by short hairpin RNA (shRNA) or small interfering RNA (siRNA).³³⁹ In the previous sections, the applications of supramolecular host–guest interaction for drug or gene delivery were discussed individually. The drug/gene codelivery systems fabricated by the host–guest interaction are summarized in this section.

Ma and co-workers have made considerable contributions to build supramolecular nanoparticles and vesicles for biomedical applications. Their work includes the drug or gene delivery, which was discussed in previous sections. Moreover, they developed multifunctional polymeric nanoparticles for drug/gene codelivery. The codelivery vehicle had core–shell structure formed via the host–guest interaction between cationic PEI polymer bearing α -CD and hydrophobic poly(benzyl L-aspartate) (PBLA) containing flanking benzyl groups. The formed core–shell nanoparticles had a hydrophobic internal core, which served as nanocontainer for hydrophobic drug storage. The outside surface showed hydrophilic property with strong positive charge capable of condensing pDNA to achieve transfection/expression efficiency in osteoblast cells. The multifunctional nanoparticles could simultaneously deliver anticancer drugs and genes, like DNA and siRNA, thus providing a new delivery vehicle for drug/gene codelivery (Figure 28).³⁴⁰ Although the gene transfection efficacy and cytotoxicity of the

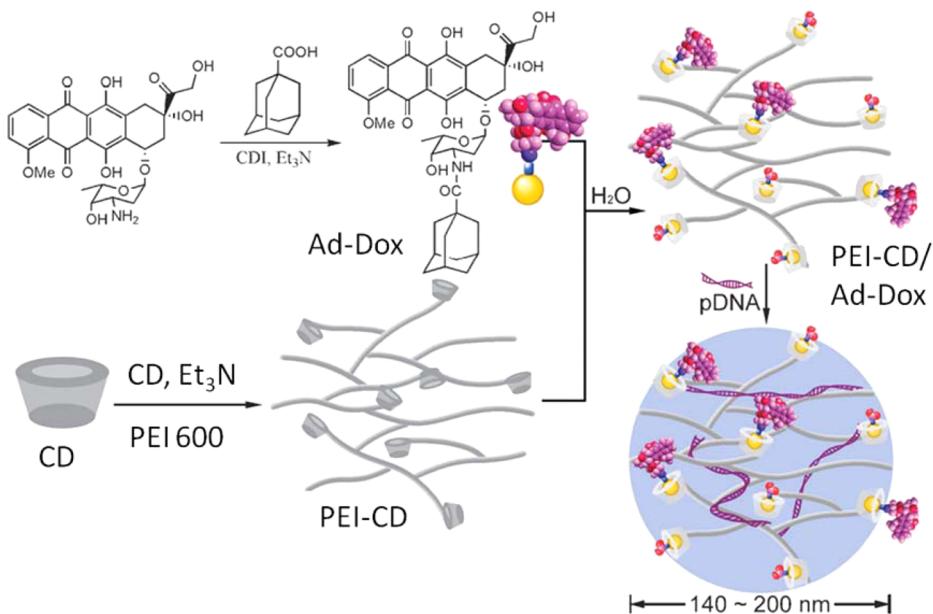


Figure 29. Preparation of PEI-CD/Ad-Dox supramolecular nanoparticles conjugated with DNA plasmid (pDNA). Reprinted with permission from ref 342. Copyright 2011 The Royal Society of Chemistry.

codelivery carrier were evaluated, its synergistic cancer therapy effect was not studied.

Supramolecular self-assemblies usually have many functional sites via noncovalent interactions, allowing for anticancer drug encapsulation and gene condensation. In supramolecular drug/gene codelivery systems, electrostatic interactions between polycations and negatively charged genes were mainly employed as a driving force for the gene condensation. On the other hand, the drug loading methods are varied. One of the loading routes is to utilize the host–guest interaction between macrocyclic molecules and anticancer drugs. For example, Yang and co-workers fabricated a pH-responsive drug/gene codelivery system by utilizing boronic acid coupling and host–guest inclusion for the cancer treatment. The anticancer drug Dox was loaded into the delivery system through the host–guest interaction with α-CD.³⁴¹ This system demonstrated excellent capability for serum-tolerant gene transfection as well as for hydrophobic drug loading and release in an acid-responsive manner. The α-CD ring was attached onto boronic acid-modified PEI (1.8k Da) through boronate linkage at neutral conditions. The electrostatic interaction between positively charged PEI and plasmid DNA led to the nanocomplex formation. The surface surrounded by the α-CD rings was believed to biomimic carbohydrate-rich cell surface, which could overcome traditional serum-susceptible drawbacks in the gene delivery. The codelivery system was found to show 25-fold higher luciferase expression as compared to the case of PEI (25k Da) in the presence of 30% serum using 293T cell line. Meanwhile, anticancer drug Dox could be quickly released upon acid-triggered disassociation of boronate linkage. Although synergistic cancer treatment was not mentioned, this work presented a novel approach for drug/gene codelivery, capable of overcoming serum-related gene delivery barriers.

In another case, Tang and co-workers have developed a series of drug/gene codelivery systems based on supramolecular nanoparticles. In 2011, they employed the host–guest interaction between β-CD and Ad to fabricate a codelivery nanocarrier (Figure 29).³⁴² The anticancer drug Dox was not directly encapsulated into the cavity of β-CD. Instead, Ad

conjugated Dox (Ad-Dox) was attached onto β-CD conjugated PEI (PEI-CD) for Dox loading via the CD-Ad complexation. The process was followed by the addition of pDNA to induce the formation of supramolecular nanoparticles with sizes of about 140–200 nm. Meanwhile, positively charged supramolecular nanoparticles could prevent pDNA from degradation. In addition to delivering anticancer drug Dox into B16F10 cancer cells, the codelivery system also successfully transfected EGFP plasmid on B16F10. In vivo study of the drug/pDNA codelivery was carried out using mice. In this work, however, synergistic effect of chemotherapy and gene therapy was not reported. Afterward, a human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-encoding plasmid gene, pTRAIL, was loaded into the supramolecular drug/gene codelivery system. pTRAIL served as a therapeutic gene to replace the model gene EGFP in their previous report.³⁴³ Similarly, the formation of drug/gene-loaded supramolecular nanoparticles was driven by the host–guest interaction as well as the electrostatic interaction. A systematic investigation on synergistic cancer therapy effect was carried out in vivo, indicating effective inhibition of the ovarian tumor growth and prolonged survival time of the tumor-bearing mice.

During the same period, another work using a similar strategy was reported, where different anticancer drug and therapeutic gene were delivered.³⁴⁴ In this case, hydrophobic anticancer drug PTX was conjugated with Ad (Ad-PTX). Supramolecular micelles were formed by the host–guest interaction between Ad-PTX and PEI-CD assisted by ultrasound technique. Therapeutic gene survivin shRNA was attached onto the positively charged surface of the supramolecular micelles. The delivered shRNA could suppress the expression of survivin in SKOV-3 cells, and further led to the reduction in the expression of Bcl-2 protein, an antiapoptotic protein mediating cell apoptosis. The silence of these two critical proteins greatly enhanced the sensitivity of tumor cells toward traditional anticancer drug, resulting in higher cancer-killing efficacy in vitro. The synergistic treatment with anticancer drug PTX and shRNA achieved effective tumor growth inhibition in vivo.

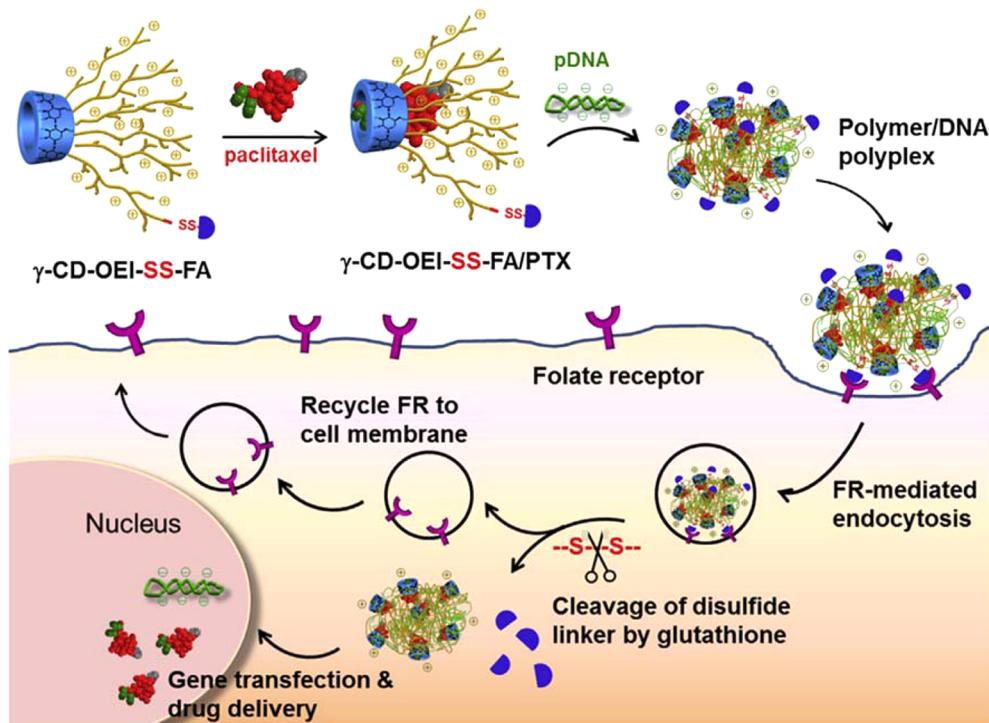


Figure 30. Drug and gene codelivery mediated by the multifunctional supramolecular self-assembly. Reprinted with permission from ref 345. Copyright 2014 Elsevier.

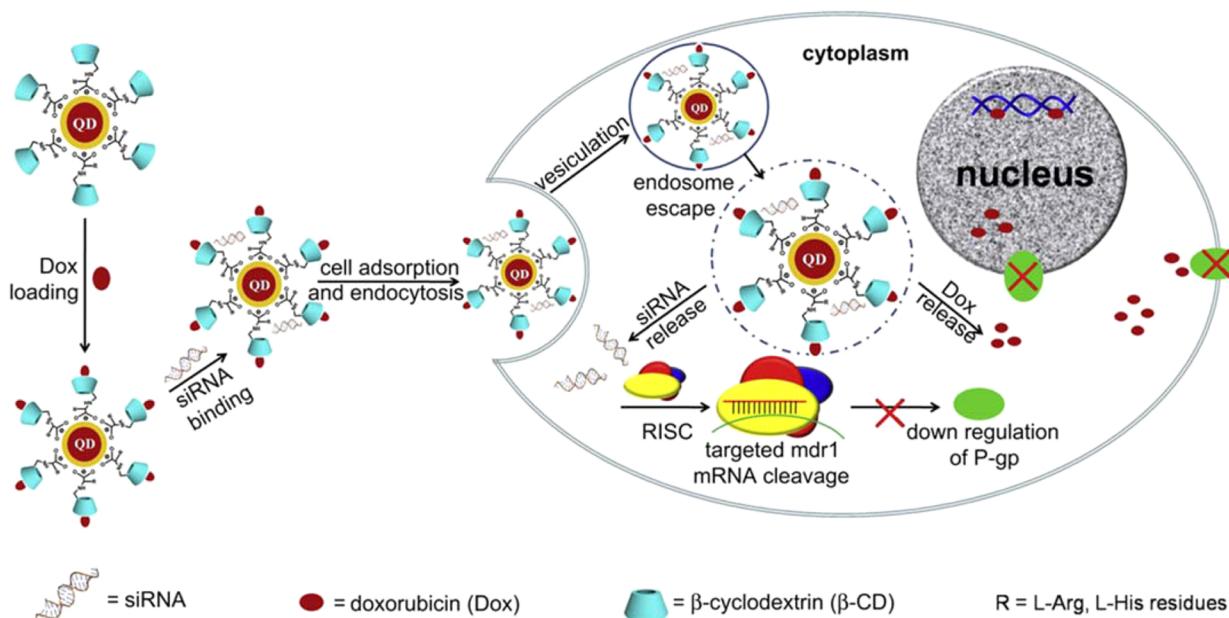


Figure 31. Preparation of multifunctional QDs as a codelivery platform for siRNA and Dox delivery to reduce multidrug resistance in cancer cells. Reprinted with permission from ref 349. Copyright 2012 Elsevier.

As previously discussed, Li and co-workers reported a targeted gene delivery system based on folic acid functionalized cationic γ -CD-oligoethylenimine star-shaped polymer (γ -CD-OEI-SS-FA). Afterward, they simply utilized the same system to simultaneously deliver hydrophobic anticancer drug PTX and cancer-therapeutic gene p53, in which the codelivery system took advantage of the host–guest interaction between γ -CD and PTX for anticancer drug loading and delivery (Figure 30).³⁴⁵ Negatively charged plasmid DNA (p53 gene) then interacted with the cationic star-shaped polymer, forming γ -CD-OEI-SS-

FA/PTX/p53 to serve as the codelivery system. The disulfide bond linked folic acid provides the targeted delivery toward KB cancer cells overexpressing folic acid receptors. The codelivery system exhibited effective delivery efficacy of wild-type p53 gene into KB cancer cells. By combining with PTX induced cell apoptosis, the codelivery system showed a promising application for cancer treatment by synergistic therapeutic effect. Another star-shaped polymer for the drug/gene codelivery was reported by Zhang and co-workers. In their early report, positively charged PAMAM dendrimer was conjugated with β -CD. Hydrophobic

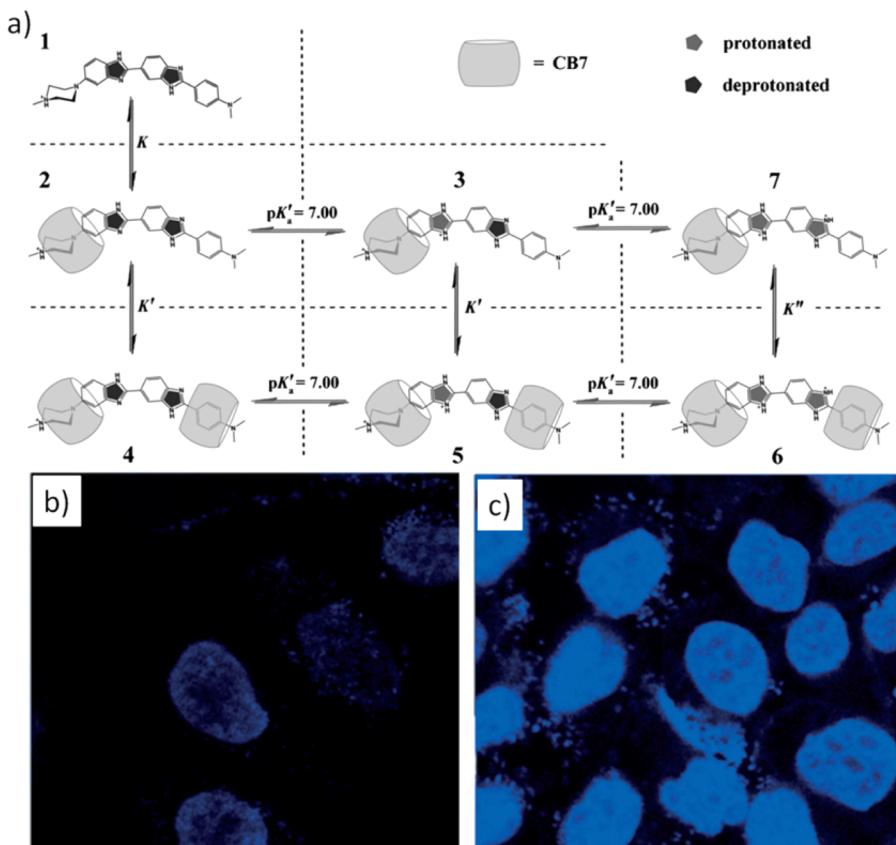


Figure 32. (a) Six major complexes formed between Hoechst 34580 and CB[7] in PBS solution (5 mM, pH 7.0) showing the protonation equilibrium and the host–guest complexation equilibrium. Confocal microscopy images of MCF-7 cells incubated with (b) 5 mM Hoechst 34580 alone or (c) 5 mM Hoechst 34580 and 50 mM CB[7]. The fluorescence images were excited at 408 nm. Reprinted with permission from ref 350. Copyright 2011 Wiley-VCH Verlag.

drug MTX was encapsulated into the cavity of β -CD for sustained release. Supramolecular micelles were formed after the siRNA condensation process. The siRNA transfection toward fibroblast cells was evaluated.³⁴⁶ They then replaced PAMAM with cationic poly(L-lysine) dendron to prepare another star-shaped system for the drug/gene codelivery. In this system, the micelle formation process was not necessary for the MTX and plasmid DNA loading.³⁴⁷ Very recently, they utilized the star-shaped system to deliver docetaxel and siRNA plasmid targeting MMP-9 (pMR3) simultaneously for the cancer treatment, which showed significant apoptosis of HNE-1 cells as compared to the case only treated by pMR3.³⁴⁸

In previously discussed codelivery systems, the electrostatic interaction between gene and cationic polymer leads to the formation of the delivery vehicles. In a different aspect, Li and co-workers developed a drug/gene codelivery system by using multifunctional QDs as the cores, which were functionalized with amino acid (L-Arg or L-His) linked β -CD (Figure 31).³⁴⁹ The siRNA binding onto the codelivery system was based on the electrostatic interaction with positively charged amino acids. An siRNA targeted to *P*-glycoprotein (*P*-gp) gene that is responsible for multidrug resistance (MDR1) via the drug efflux mechanism was chosen. Anticancer drug Dox was encapsulated into the hydrophobic cavity of β -CD, which could bypass the *P*-gp mediated drug efflux. Upon the administration of the codelivery system containing Dox and siRNA against *P*-gp protein into HeLa cells, the MDR1 mRNA level and related *P*-gp protein expression were significantly suppressed. Because of the down-regulation of *P*-gp, enhanced internalization of Dox inside HeLa

cells was observed. The synergistic therapeutic efficacy was substantially enhanced when compared to the case treated with free Dox only. In addition, the authors compared the siRNA delivery efficacy by using two different amino acids, and observed superior siRNA delivery efficacy by L-Arg containing codelivery system due to its stronger positive charge. Another advantage of the QD-based codelivery system was that the inherent fluorescence property of QDs could provide a facile method for real-time intracellular tracking of the codelivery vehicle. In addition to employing functionalized QDs as the codelivery system, Ma and co-workers reported redox-responsive drug/siRNA codelivery carrier based on MSNPs.²³²

Unlike the above-mentioned codelivery systems that mainly utilized the host–guest interaction to encapsulate anticancer drugs, mesoporous pores of MSNPs were used to load anticancer drug Dox in this case. Disulfide bond linked Ad on the nanoparticle surface could effectively complex with amino β -CD via the host–guest interaction, which could block the loaded Dox within the mesopores to prevent the prerelease. The amino groups modified on β -CD were used to complex with siRNA against antiapoptotic protein Bcl-2. Upon entering into cancer cells, the disulfide bond could be effectively cleaved by intracellular reduction provided by a high concentration of intracellular GSH, resulting in the uncapping of amino β -CD for the drug/siRNA corelease. In vitro study showed high cancer-therapy efficacy when delivering Dox and siRNA into HeLa cells simultaneously. In vivo study using zebrafish models demonstrated significant tumor growth inhibition from delivered

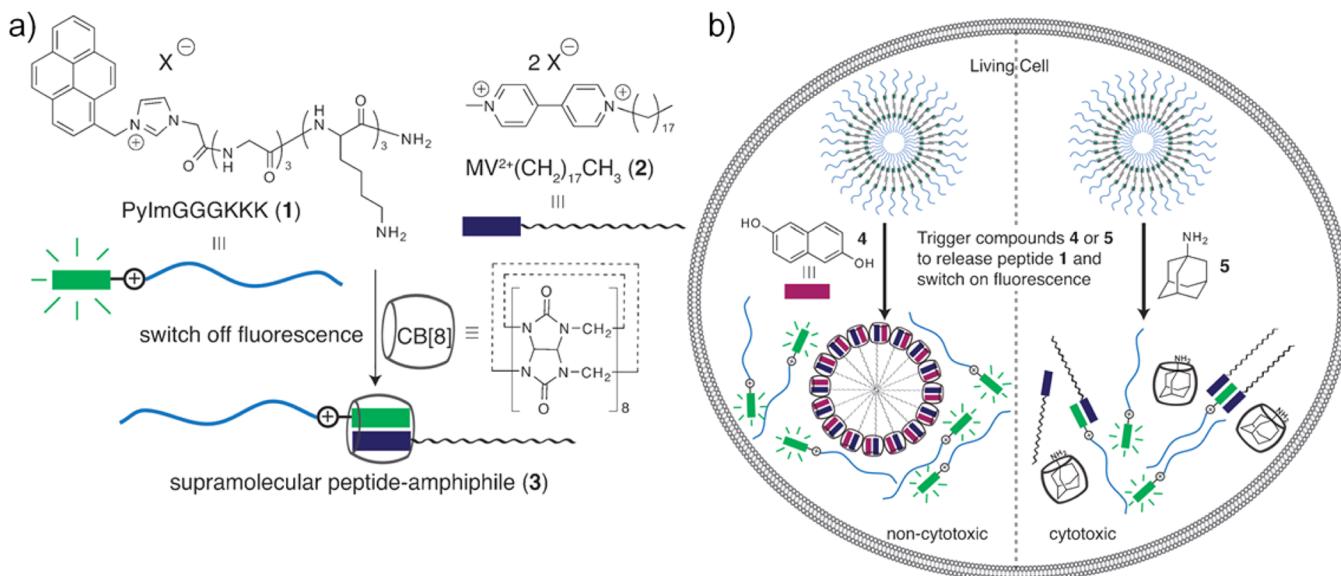


Figure 33. (a) Chemical structures of pyrene imidazolium-labeled peptide and viologen-functionalized lipid, and the formation of ternary complex with CB[8]. The ternary complex further self-assembles to form supramolecular vesicles with the “switch-off” of the fluorescence. (b) Peptide release with the “switch-on” of the fluorescence through the dissociation of the host–guest complex upon the addition of external competitive guest 4 or 5. Reprinted with permission from ref 357. Copyright 2012 Wiley-VCH Verlag.

anticancer drug Dox, as well as high gene silence capability from delivered siRNA against GFP expression.

Drug/gene codelivery emerging as a novel strategy for cancer treatment has drawn considerable attention in biomedical field. Utilizing supramolecular strategy to fabricate drug/gene codelivery systems exhibits several advantages such as easy fabrication and controllable drug/gene loading. In recent years, researchers have successfully employed the host–guest interactions to develop drug/gene codelivery systems, which are in fact the extension of either drug or gene delivery systems in their previous studies. Interestingly, the synergistic therapeutic effect of delivered drug/gene presents a promising future for their clinical application in cancer treatment.

2.4. Bioimaging

Biological imaging provides necessary information for the diagnostics of diseases. An ideal bioimaging reagent should meet several requirements, including good biocompatibility, high stability against biochemical degradation and photobleaching, as well as strong signal generation capability. The major purpose of applying supramolecular chemistry in bioimaging is to improve the above-mentioned properties of bioimaging reagents to achieve successful visualization of target cells or tissues without inducing unwanted side effects or toxicity to the biological systems.

Direct host–guest complexes between macrocyclic molecules and fluorescence dyes have been utilized for bioimaging, showing tunable or stimuli-responsive fluorescence emission, physiochemical shielding, as well as enhanced biocompatibility provided by macrocyclic host molecules. Nau and co-workers contributed a comprehensive review article about the host–guest complexes formed between fluorescence dyes and macrocyclic molecules in aqueous environment.³⁵¹ Montes-Navajas and co-workers proved the cell membrane penetrating capability and cellular uptake of the host–guest complexes between CBs and two fluorescence dyes, acridine orange and pyronine Y.³⁵² Typical nucleus staining fluorescence dyes, including Hoechst 34580 and 33250, exhibited strong fluorescence when binding onto the

DNA groove, the interaction of which might affect the normal metabolism process of cells. Researchers took advantage of the host–guest complexation between CBs and fluorescence dyes to overcome such a drawback in biological imaging. The host–guest interaction between CB[7] and Hoechst dyes for fluorescence emission enhancement and responsiveness to external pH changes was investigated (Figure 32a).^{353,354} In vitro biological imaging then was carried out using breast cancer MCF-7 cell line. As compared to the same amount of Hoechst 34580 used, the host–guest complex between Hoechst 34580 and CB[7] showed much stronger fluorescence intensity from the cell nucleus (Figure 32b,c). In addition, the host–guest complex was capable of improving the photostability and decreasing the DNA binding affinity.³⁵⁰ Fluorescent supramolecular nanoparticles can be produced by introducing fluorescence dyes into the building blocks via covalent linkage or host–guest complexation. Tseng and co-workers developed a novel technique based on microfluidic droplet generator to produce fluorescent supramolecular nanoparticles for targeted cell imaging. The formation of supramolecular nanoparticles was driven by the host–guest inclusion between β-CD and Ad, which were separately conjugated onto PEI and PAMAM. Fluorescence dye Cy5 was attached onto the CD-PEI conjugate. In vitro cellular uptake and targeted fluorescence imaging were carried out with U87 and MCF-7 cell lines.³⁵⁵ In another case, Xu and co-workers prepared fluorescent supramolecular nanoparticles by the self-assembly of amphiphilic β-CD modified fluorine copolymer, noted as PFC6CD. The host–guest inclusion between β-CD and various guest molecules led to different fluorescence spectra of the supramolecular nanoparticles, which could be used for organic molecule sensing and cell labeling. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity assay and in vitro fluorescence imaging of the supramolecular nanoparticles were carried out with KB cells, revealing good biocompatibility and effective cellular uptake of the supramolecular nanoparticles.³⁵⁶

Supramolecular vesicles not only serve as the delivery carriers by loading drugs inside the hollow cores, but also provide

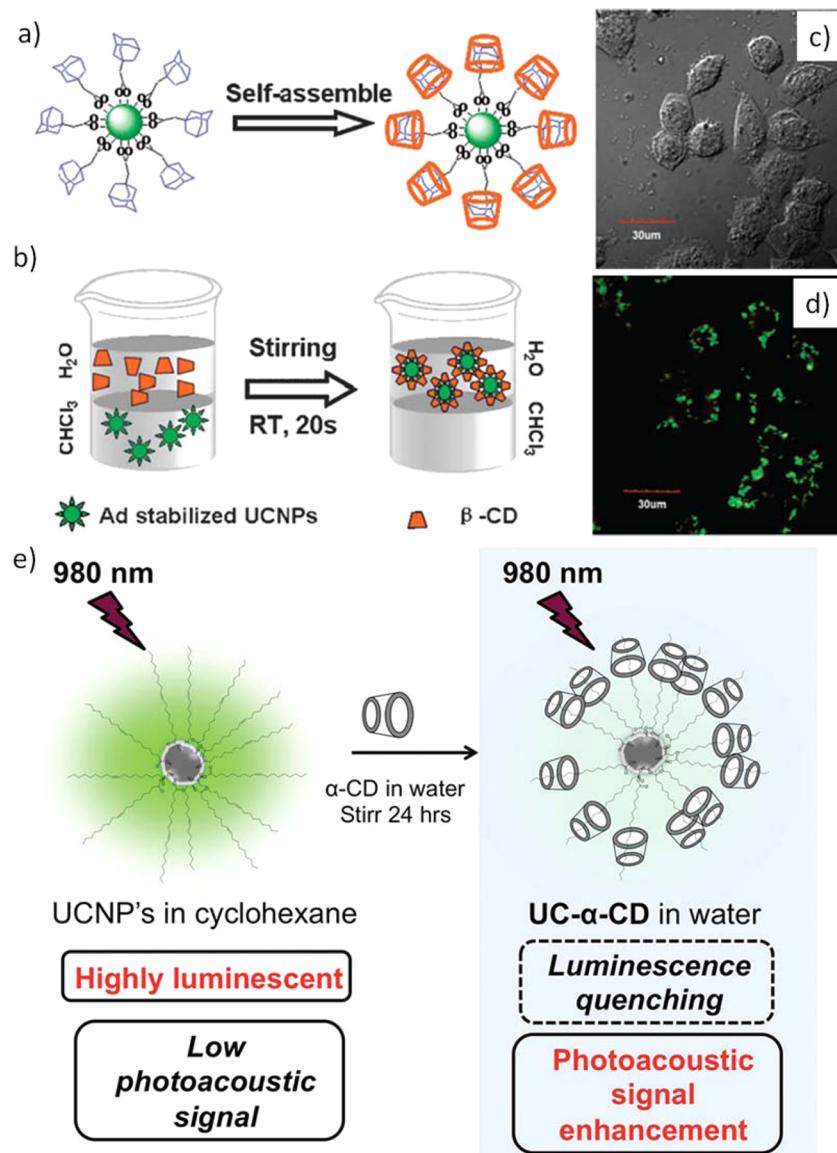


Figure 34. (a) Self-assembly and (b) phase transfer of UCNPs-Ad from chloroform phase to aqueous phase through the interaction between β -CD and Ad. (c) Bright-field and (d) luminescence images of KB cells stained with 200 mg mL⁻¹ UCNPs-Ad/ β -CD for 2 h. Reprinted with permission from ref 363. Copyright 2010 The Royal Society of Chemistry. (e) Schematic illustration of luminescence quenching effect and subsequent photoacoustic signal enhancement from UC- α -CD in water. Reprinted with permission from ref 364. Copyright 2014 Wiley-VCH Verlag.

fluorescence labeling in biological systems. For example, Scherman and co-workers reported supramolecular vesicles consisting of amphiphilic pyrene-viologen-CB[8] ternary complex (Figure 33).³⁵⁷ The complex between pyrene imidazolium-labeled peptide and CB[8] was formed via the host–guest inclusion as the hydrophilic segment. Viologen lipid acted as the hydrophobic segment in the ternary complex. The supramolecular micelles could effectively enter into living HeLa cancer cells, accomplishing intracellular peptide delivery. Upon the addition of competitive guest molecules, the peptide could be released inside cells to simultaneously induce the “switch-on” of fluorescence as well as the cytotoxic effect toward cancer cells. The system successfully presented dynamic and responsive behavior of the host–guest complexes inside living cells. The on-demand activation of the peptide release, “switch-on” fluorescence, and toxicity effect provide a facile approach for the fabrication of the next generation theranostic platforms.

Pillar[n]arene is a new type of macrocyclic molecules with great potential in biomedical applications.^{358,359} Zhang and co-workers fabricated a thermoresponsive fluorescence vesicle based on pillar[5]arene for intracellular imaging.³⁶⁰ They then further developed an amphiphilic pillar[5]arene to construct supramolecular vesicles for delivering mixed dyes. The supramolecular vesicles exhibited good biocompatibility toward HeLa cell line. The bioimaging by dual fluorescence emission from delivered FITC and Rhodamine B was accomplished inside HeLa cells.³⁶¹ In a recent report, they anchored water-soluble pillar[5]arene onto graphene oxide and reduced graphene oxide to enhance their water solubility. Fluorescent guest molecules then were introduced into the hybrid materials via the host–guest inclusion with pillar[5]arene for the purpose of in vitro fluorescence imaging. In addition, the anchored pillar[5]arene provided functional sites for facile functionalization on the hybrid materials. Taking advantage of the Raman signal from grapheme

oxide, the hybrid was also employed as a Raman imaging reporter in HeLa cells.³⁶²

Noble metal nanoparticles with excellent optical properties show great potentials for bioimaging applications, in virtue of their strong signals and nanoscale size. However, their toxicity and poor water solubility have greatly hampered their biological usage, especially for *in vivo* applications. Supramolecular chemistry has been successfully utilized to resolve these problems. For example, upconversion nanoparticles (UCNPs) can serve as an excellent bioimaging probe on account of their NIR absorption and upconversion emission. To achieve uniform UCNPs with good water solubility, Liu and co-workers used hydrophobic group Ad to stabilize UCNPs in organic solvent, CHCl₃.³⁶³ By simple addition of β -CD to effectively form the host–guest complex with Ad, the functionalized UCNPs became water-soluble due to the coverage of hydrophilic β -CD (Figure 34a–d). The water-soluble UCNPs were quickly pulled into the water phase within 20 s under mild stirring at room temperature. This method provides a facile approach to convert hydrophobic UCNPs into hydrophilic UCNPs, which were found to be biocompatible, exhibiting bright upconversion fluorescence inside KB cells. To generalize this strategy for easily converting hydrophobic nanoparticles into hydrophilic ones, the authors further used α -CD to form the host–guest complex with oleic acid (OA) capping ligand that was attached on the UCNPs surface.³⁶⁵ The interesting advantage of this system was that the hydrophilic UCNPs obtained by the α -CD complexation could further host hydrophobic cargos, like osmium(II) complex, showing a possibility for hydrophobic drug delivery. The authors carried out the positron emission tomography (PET)-based bioimaging using the developed hydrophilic UCNPs by interaction with ¹⁸F. *In vivo* PET imaging of whole-body small animals by the hydrophilic UCNPs was investigated. The hydrophilic UCNPs could realize bioimaging by both luminescence and fluorescence at the same time, serving as a multiple imaging probe. Very recently, Zhao and coauthors extended this strategy for photoacoustic imaging in living mice (Figure 34e).³⁶⁴ Threading of α -CD onto the surface of UCNPs led to increased water solubility of the system, while photoluminescence was decreased. Instead of sticking to photoluminescence, photoacoustic property of UCNPs was utilized to visualize the distribution of UCNPs within living mice.

In addition to the optical signals such as luminescence and fluorescence, magnetic resonance imaging (MRI) technique was also widely used for bioimaging. Biocompatible MRI contrast agents with significant signal amplification are always desired for ideal MRI imaging. Liu and co-workers constructed a supramolecular polymer based on Mn-porphyrin bearing PEG (Mn-TPP) and bis(permethyl- β -CD). The host–guest interaction between CD and the TPP moiety acted as the linkage for the supramolecular polymer formation. The cavity of β -CD could effectively prevent Mn-TPP from the oxidization, resulting in higher electronic spin. Introduced PEG section in the supramolecular polymer was beneficial to improve the blood circulation time, and to enhance the biocompatibility of the supramolecular polymer-based MRI agent. *In vivo* MRI imaging investigation certified the enhancement of the MRI signal within different organs of mice.³⁶⁶

Bioimaging technique plays a critical role in disease diagnosis. Developing biocompatible, sensitive, and precise bioimaging reagents has been a long-cherished goal in the biomedical field. Upon the advancement of novel nanomaterials with superior optical or magnetic properties, how to safely utilize them in the

biological environment for bioimaging becomes a new research topic. Host–guest interactions have been appropriately employed to address such a problem in biomedical field and showed unique advantages. Thus, a growing interest in this research topic will lead to a fast development of the field.

2.5. Photodynamic Therapy

PDT is a noninvasive cancer treatment technique, which has attracted significant attention in the past few decades, because of using nontoxic components and controllable administration by tunable light irradiation. Generally, PDT requires three components, including photosensitizer (PS), oxygen, and light irradiation with proper wavelength. The success of photodynamic cancer therapy is based on the prerequisite of effective accumulation of enough PS in the target cancer cells or tumor sites. Upon light irradiation with proper wavelength, the PS can utilize the light energy to convert oxygen (³O₂) in the surrounding environment into highly toxic singlet oxygen (¹O₂). Considering the bioavailability of oxygen and easy treatment of light, the major barrier in PDT is effective delivery of PS without losing its photodynamic activity. For example, although many PSs show efficient singlet oxygen conversion capability, their biological applications in PDT were hampered by their poor water solubility and aggregation-induced photodynamic activity quenching.^{367–369} Generally, researchers directly conjugated water-soluble moieties, like PEG, onto PS molecules to enhance their water solubility, the strategy of which is time-consuming and may be accompanied by a difficult purification process. Thus, supramolecular chemistry technique stands out as a new method for addressing such issues in photodynamic cancer therapy.

Porphyrin and its derivatives are one kind of important PS widely used in PDT for cancer treatment. In aqueous solution, however, due to strong hydrophobic π – π interaction, porphyrins tend to aggregate, resulting in the self-quenching of their excited state and thus losing the photodynamic property. In this case, singlet oxygen generation capability and phototoxicity effect are severely decreased. To dissociate the self-aggregates of porphyrins in water, Kano and co-workers fabricated octaarginine peptide conjugated meso-tetraphenylporphyrin (R8-TPP) and heptakis(2,3,6-tri-O-methyl)- β -CD (TMe- β -CD). The host–guest complex (2:1) between β -CD and the hydrophobic moiety of phorphyrin in R8-TPP could greatly stabilize the porphyrin derivative. Cellular uptake of R8-TPP was enhanced more than twice by adding TMe- β -CD to form the water-soluble complex. The addition of TMe- β -CD also altered the cellular uptake mechanism and lysosome escape efficacy of R8-TPP, leading to higher photocytotoxicity upon the same amount of light irradiation in HeLa cancer cell line.³⁷⁰ Apart from using CD to host PS, methylene blue (MB), a photosensitizer, was encapsulated into the cavity of CB[7], forming a host–guest complex (1:1) with high binding constant ((1.26 ± 0.28) × 10⁷ M⁻¹ in water).³⁷¹ The aggregation of MB was avoided by such encapsulation even at high concentration, and CB[7] could also protect MB from oxygen attack, reserving its photodynamic activity. The rate of singlet oxygen generation from the MB-CB[7] host–guest complex was investigated. Although delayed singlet oxygen generation was revealed, this work still showed the significance of using the host–guest complex for reserving PS for PDT.

As discussed above, PS-containing host–guest complex not only effectively prevents hydrophobic PS from aggregation-induced photodynamic activity quenching, but also enhances the

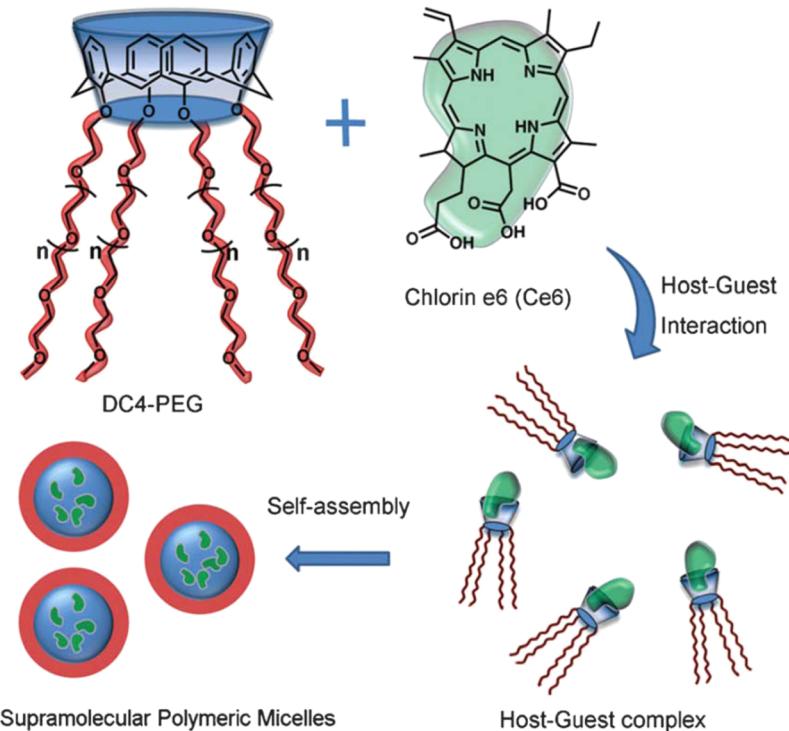


Figure 35. Chemical structure of star-like calix[4]arene host, Chlorin e6 guest, and supramolecular polymeric micelles formed on the basis of the host–guest interaction. Reprinted with permission from ref 372. Copyright 2011 The Royal Society of Chemistry.

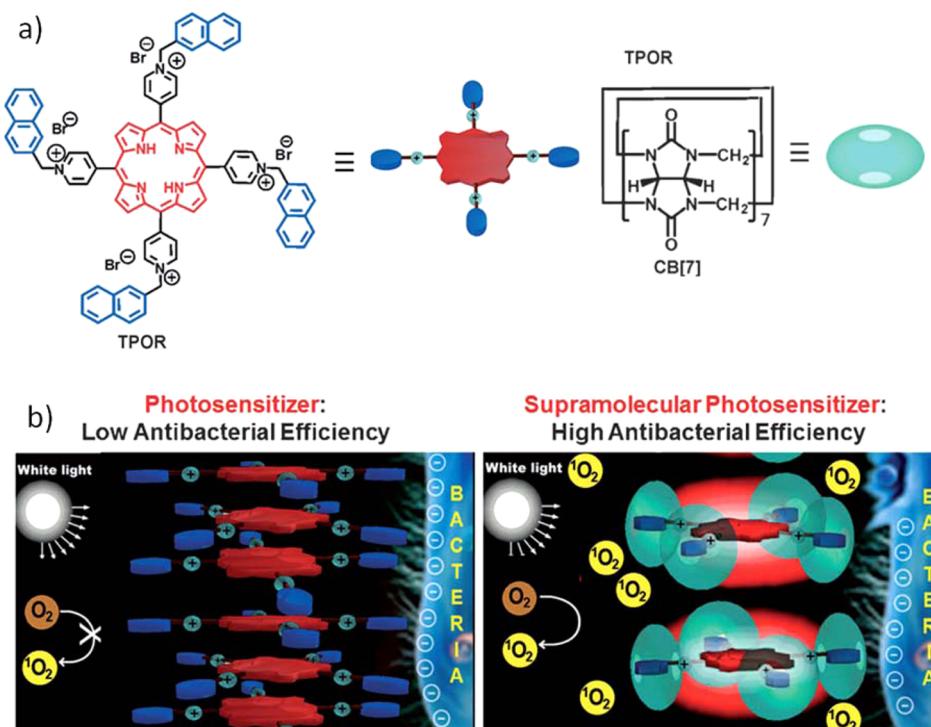


Figure 36. (a) Chemical structures of photosensitizer TPOR and CB[7]. (b) The construction of TPOR/(CB[7])₄ supramolecular photosensitizer and the mechanism for the enhanced antibacterial efficiency of TPOR/(CB[7])₄ as compared to that of TPOR. Reprinted with permission from ref 373. Copyright 2013 Wiley-VCH Verlag.

water solubility of PS for PDT application in biological environment. In another report, instead of directly functionalizing PEG onto PS to improve its water solubility, water-soluble PEGylated star-like calix[4]arene derivative was prepared (Figure 35).³⁷² By utilizing strong host–guest interaction, a

hydrophobic PS, Chlorin e6 (Ce6), could be encapsulated into the hydrophobic cavity of the calix[4]arene derivative. The resultant 1:1 host–guest complex, calix[4]arene-Ce6, could prevent Ce6 from self-aggregation and fluorescence self-quenching, in which way photodynamic activity of Ce6 was

reserved. Moreover, the calix[4]arene-Ce6 host–guest complex could further self-assemble into supramolecular polymeric micelles with uniform size of about 200 nm (Figure 2S). Because of the improved cellular uptake capability and reserved photodynamic activity of hydrophobic PS, enhanced photocytotoxicity of the supramolecular micelles carrying hydrophobic Ce6 in HeLa cancer cells was observed by comparing to the treatment of free Ce6.

Another solution to solve the aggregation problem of PSs was to use macrocyclic molecules, such as CB[7], as bulky building blocks to construct supramolecular PSs.³⁷³ Initially, a porphyrin was functionalized with positively charged naphthalene-methylpyridinium terminals (TPOR). The construction of supramolecular PS was easily accomplished by the addition of CB[7] into an aqueous solution containing TPOR (Figure 36). The binding constant between CB[7] and naphthalene-methylpyridinium unit was calculated to be 6.6×10^{-7} , which provided sufficiently high driving force for the 4:1 host–guest complex formation between CB[7] and TPOR. The formed supramolecular PSCB[7]-TPOR exhibited excellent antibacterial efficacy by generating toxic singlet oxygen. As compared to the same amount of free TPOR, the inhibition ratio toward *E. coli* increased from 17% to 97%. Another advantage of using supramolecular PS was the enhanced biocompatibility by using biologically environmentally friendly CB[7] to encapsulate toxic organic species.

The concept of employing the interlacing spacer to separate hydrophobic PS was realized inside the nanochannels of MSNPs,³⁷⁴ where hydrophobic Ad group was chemically modified onto the internal surface of the mesopores in MSNPs, acting as an interlacing spacer to separate hydrophobic zinc phthalocyanine (ZnPc), a second generation of PS with high singlet oxygen generation efficiency upon red light irradiation. Hydrophobic/hydrophilic interaction could lead to automatic disassembly of ZnPc at its monomer state in the nanochannels. In aqueous solution, the ZnPc could be prevented from $\pi-\pi$ stacking interaction-induced aggregation and retain its singlet oxygen generation capability. On the other hand, the hydrophobic outside surface of MSNPs brought by chemically conjugated Ad unit greatly limited the cellular uptake efficiency. By adding amino-modified β -CD to form the host–guest complex with Ad, the chemical property of the outside surface was readily changed, leading to enhanced cellular uptake of ZnPc loaded MSNPs favorable for PDT. In this MSNP-based PDT system, the host–guest interaction was used to improve the biocompatibility of the system.

Using the host–guest interactions to resolve low water-solubility and photodynamic quenching issues of hydrophobic PSs is a relatively new research topic in the biomedical field. Current studies have proven the feasibility and advantages of such applications. Specific targeting toward cancer cells and the stability of host–guest interaction-based photodynamic therapeutic systems still require more investigations.

3. CONCLUSIONS AND PROSPECTS

Ever since the discovery of supramolecular chemistry, it has been applied in many research fields. During the past two decades, tremendous efforts have been dedicated to biomedical applications of supramolecular systems based on noncovalent interactions, including hydrogen-bonding interaction, electrostatic interaction, van der Waals forces, hydrophobic/hydrophilic interaction, host–guest interaction, and so forth. In virtue of the reversibility and adaptability of host–guest interaction, supramolecular systems have been evolved into various morphologies with different functions to fulfill specific requirements in biomedical applications. Taking account of the relatively high popularity in biological applications, three major macrocyclic molecules, CDs, CAs, and CBs, were highlighted in this Review. Thus, this Review covered current research progress of host–guest supramolecular systems in biomedical applications, mainly addressing drug delivery, gene delivery, bioimaging, and photodynamic cancer therapy.

The biomedical application of macrocyclic molecules started from simple usage of host–guest complexes, which not only enhance water solubility of drugs, but also alter their release rate and bioavailability. Through chemical functionalization, these macrocyclic molecules were endowed with new properties, such as high water solubility and biocompatibility. Cationic macrocyclic molecules were prepared by conjugating positively charged functional groups for the purpose of gene condensation. To prepare supramolecular vesicles or micelles for the drug delivery, amphiphilic macrocyclic molecules were fabricated by conjugating with different moieties, such as hydrophilic and hydrophobic groups. The host–guest complexation provides the connection for the formation of supramolecular nanoparticles through the self-assembly, as well as a facile approach of functionalization on supramolecular nanoparticles. Responsive polymer hydrogels based on reversible host–guest complexes could be employed for injectable drug delivery. Polyrotaxanes prepared by threading macrocyclic molecules onto polymer chains were used for the construction of supramolecular nanoparticles and hydrogels. In some cases, polyrotaxanes could be directly used for drug and gene delivery. By combining the host–guest complexes with novel nanomaterials, new nanohybrids were obtained for biomedical applications. For instance, “on–off switch” on MSNPs was constructed by anchoring the host–guest complex-based nanogate at the pore orifice of MSNPs, aiming at controlled drug release. By introducing the host–guest interactions on the nanoparticle surface, multifunctional theranostic platforms were achieved. In most cases, the application of supramolecular systems in gene delivery was based on the electrostatic interaction between cationic moieties and negatively charged gene for gene condensation. Either electrostatic interaction or host–guest complexation was responsible for the formation of supramolecular self-assembly to serve as nanosized gene carriers. Host–guest complexation was also used to protect bioimaging agents and photosensitizers, including enhancing their stability and improving their fluorescence or photodynamic activity in biophysical environment.

Along with rapid advancement of supramolecular chemistry and new functionalization techniques, the host–guest complexes with multifunctional properties have shown great potentials for biomedical applications. Host–guest interactions provide numerous strategies to fabricate multifunctional supramolecular systems. Although a large amount of scientific studies on biomedical applications of supramolecular systems have been published, clinical usage of such supramolecular systems is still facing many practical challenges, including long-term toxicity effect brought by various chemical groups, immunological reaction, biodegradation, excretion, and so forth. To ensure biological safety when applying these supramolecular systems in biomedical applications, the development of biocompatible and biodegradable supramolecular systems will be a major focus in future research. Moreover, the exploration of existing supramolecular systems for clinical application is another important

task in this field, such as in-depth investigations on biological interactions between living organism and supramolecular systems. The potential toxicity of external functional moieties introduced onto the host or guest molecules needs to be investigated, which should be essentially biocompatible. From another aspect, novel theranostic platforms capable of accomplishing multiple functions are highly desired. Considering the advantage of facile fabrication in the host–guest chemistry, newly designed supramolecular systems will be beneficial to improve the capability of the current theranostic techniques. For example, the ability of simultaneous imaging or targeted navigation inside physiological environment will greatly improve the therapeutic efficiency in the biomedical systems. It can be expected that patients will benefit more from clinical theranostics based on supramolecular systems in the future.

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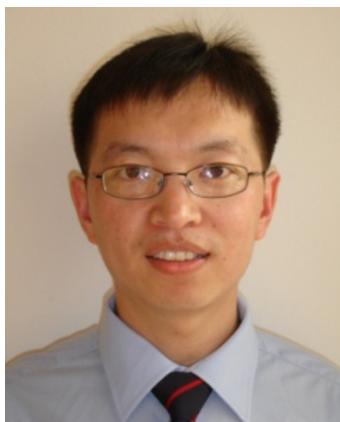
Notes

The authors declare no competing financial interest.

Biographies



Xing Ma received his B.Sc. in Materials Science and Engineering at Harbin Institute of Technology, China in 2009. He conducted his Ph.D. study at Iowa State University, IA from August 2009 to May 2010 under the supervision of Professor Mufit Akinc. Afterward, he transferred to Nanyang Technological University, Singapore in January 2011 and continued his Ph.D. research under Professor Yanli Zhao's supervision. He obtained his Ph.D. degree of Materials Science and Engineering in December 2013. He is now a postdoctoral fellow at Max Planck Institute for Intelligent Systems, Germany. He has wide research interests in biological applications of nanomaterials and supramolecular assemblies.



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