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Cyclodextrin-Based Host–Guest Supramolecular Nanoparticles for Delivery: From Design to Applications

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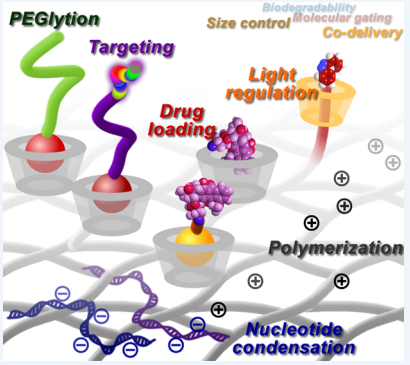
CONSPECTUS: Efficient assembly in host–guest interactions is crucial to supramolecular nanotechnology. Cyclodextrins (CDs), which possess a hydrophilic exterior surface and hydrophobic interior cavity on the truncated cone, improve the biocompatibility of nanodelivery systems, and hence, supramolecular approaches utilizing CDs can improve and expand the design and applications of functional delivery systems. Owing to good inclusion ability, αCD and βCD are commonly used in the design and construction of supramolecular structures.



In this Account, we describe the design strategies to adopt CDs in host–guest delivery systems. Modification of CDs with polymers is popular in current research due to the potential benefits rendered by cationic protection and improved capability. While the process has only minor influence on the host characteristics of the CD cavity, the interaction between the CD and the guest moiety imparts new attributes to the nanosystems with guest-decorated functional groups such as adamantyl poly(ethylene glycol) (PEG) for coating protection, hybrid guests for conformational flexibility, and adamantyl prodrugs for drug delivery. Some specific agents form inclusion complexes with the polymerized βCDs directly and core–shell nanoparticles with hydrophobic cores and are usually created to carry insoluble drugs while the hydrophilic shells offer protection. These unique designs provide the means to practically adapt special characteristics for additional functions or co-delivery.

In order to be accepted clinically, delivery systems need to possess extra functions such as controlled particle size, biodegradability, controlled release, and targeted delivery to overcome the hurdles in delivery. These features can be added to biomaterials by self-assembly of functional groups facilitated by the host–guest interactions. Size control by hybridization of switchable polymer compartments in supramolecular structures contributes to the biodistribution utility and biodegradability by incorporating the moieties with hydrolyzable connections and enhancing intracellular degradation and clearance. Controlled release by application of responsive structures like molecular gatings eased by the host–guest interaction can be triggered by the tumor microenvironment at extreme pH and temperature or by external stimuli such as light. Along with the binding selectivity and controlled release, the host–guest nanoparticles show enhanced efficacy in delivery especially to tumors. Recent developments in supramolecular co-delivery systems are described in this Account. Nanoparticles can be designed to carry adamantyl prodrugs and therapeutic nucleotides to tumors so that the released drugs and gene expression synergistically inhibit malignant tissue growth.

Optimization of nanoparticle delivery systems by multifunctional transitions yields better biocompatibility and controlled response, and such novel designs will expedite *in vivo* applications. Hence, multifunctional CD-based host–guest supramolecular nanoparticles with co-delivery ability are expected to have many potential clinical applications.

1. INTRODUCTION

Supramolecular nanotechnology has been recognized as a significant approach in the design of delivery systems. The ability to self-assemble readily allows the formation of macromolecular nanoparticles with multiple components offering the appropriate binding strength resulting from noncovalent interactions in specific circumstances. Formation of the host–guest complex requires combination of several elemental noncovalent interactions such as hydrophobic interactions and geometric fitting within the interaction structure.[1] In order to attain functional delivery, nanomaterials must possess enhanced properties. In addition to common host molecules such as crown ethers, calixarenes, cucurbiturils, pillararenes, and metallo structures,[2−8] cyclodextrins (CDs) have insignificant toxicity, and their improved bioavailability renders them suitable for functional delivery systems.[1,9] As seminatural compounds of cyclic oligosaccharides possessing molecular-compatible cavities, CDs are commonly composed of five or more α-D-glucopyranoside units in a ring linked by α-1,4-glycosidic bonds. The

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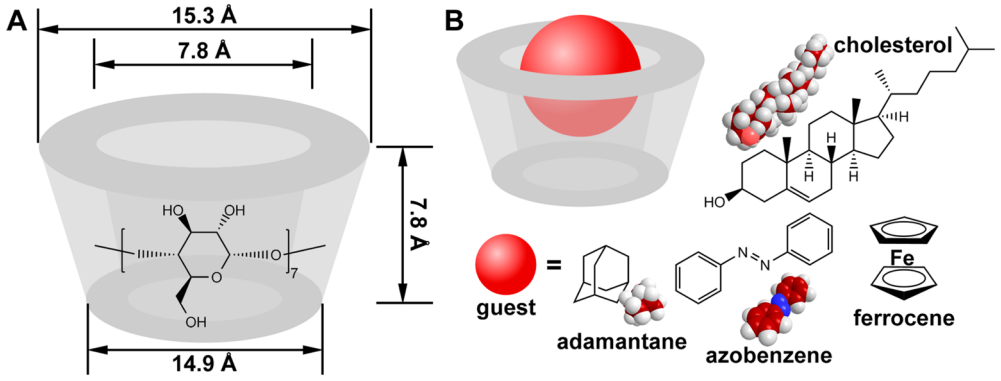

Figure 1. (A) Structure of βCD and (B) common guest molecules forming inclusion complexes with βCD host.

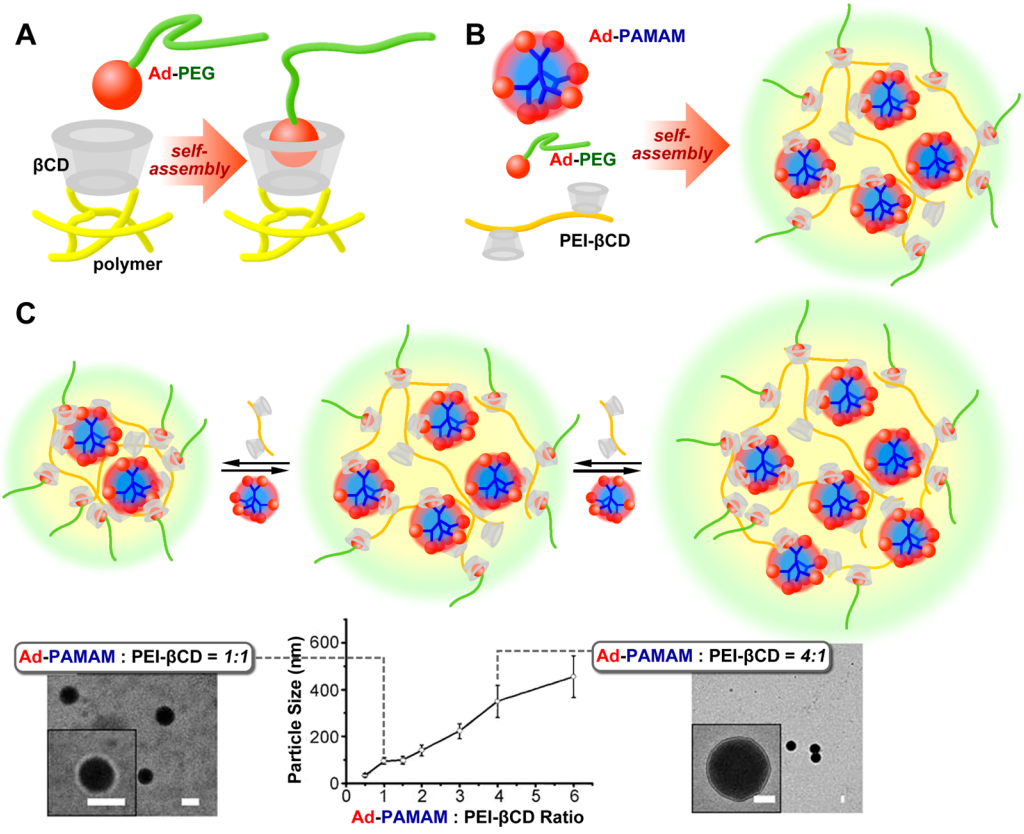

Figure 2. (A) General PEGlytion on βCD-grafted polymer mediated by adamantyl PEG (Ad-PEG). (B) Synthesis, and (C) size control of supramolecular nanoparticles assembled by PEI−βCD, Ad-PEG, and Ad-PAMAM with the "bricks and mortar" strategy, presented by electron microscopy with scale bar = 100 nm. Adapted with permission from ref 18, copyright 2009 Wiley.

frequently used CDs contain six, seven, or eight glucopyranoside monomers, namely, α-cyclodextrin (αCD), β-cyclodextrin (βCD), or γ-cyclodextrin (γCD), respectively. Generally, CDs have a truncated cone structure that has a hydrophilic exterior surface and hydrophobic interior cavity. Owing to the different inclusion ability, αCD and βCD are commonly used in the design and synthesis of supramolecular nanoparticles. In this Account, recent progress and approaches to CD-based delivery systems are described.

2. CYCLODEXTRIN CAVITY AND GUEST COMPANIONS

2.1. Common Host−Guest Structures with β-Cyclodextrins

Modification of βCD using polymers is popular in delivery systems. Polymers are usually grafted to the CDs to provide a scaffold or platform for CD molecules mostly with cationic surface charges. They also improve the delivery capability. βCD reduces the toxicity of the grafted polymer and brings additional benefits such as membrane absorption enhancement and molecular stabilization. For example, polyethylenimine (PEI), considered the gold standard of gene transfection, is a prominent cationic polymer capable of gene transfection, and CD-modified polyethylenimine derivatives possess lower toxicity than the unmodified branched or linear analogue.[10] We have developed βCD-cross-linked polyethylenimine (PEI−βCD). The PEI−βCD retains the cationic polymer feature, enhanced intracellular function for gene delivery, and significantly reduced toxicity. The PEI−βCD exhibits improved biocompatibility over nondegradable PEI with a molecular weight of 25 kDa (PEI 25 kDa).[11] Modification strategies have only minor influence on the

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CD cavity, which still has the "host" characteristics to encompass "guest" molecules such as the adamantyl group.

The noncovalent interaction between the βCD cavity and the adamantyl group is typical of the host−guest system. The hydrophobic cavity in βCD can form inclusion complexes with several guest moieties (adamantane, azobenzene, ferrocene, cholesterol, etc., as shown in Figure 1).[1] Despite the solution viscosity and size effect, the association constant of βCD and the adamantyl group of approximately 1×10^5 M^{-1} in water[1] may decrease but still leads to strong affinity in macromolecular self-assembly. The βCD@adamantane implants are useful in nanoparticle-mediated delivery systems. The most common strategy is modification of βCD-containing nanoparticles with poly(ethylene glycol) with an adamantyl end (Ad-PEG) called PEGylation (Figure 2A). The PEG chains are usually employed to increase the water solubility of the nanoparticles,[10] and Ad-PEG has an amphiphilic nature. The hydrophobic adamantyl end can be incorporated into the βCD cavity, while the hydrophilic PEG chain is located on the nanoparticle periphery. Coating PEGylation overcomes the insolubility problem while reducing the influence of serum and other environmental factors during delivery.

The advantage of the Ad-PEG guest is exploited by various nanocarriers in drug, plasmid DNA, and small interfering RNA (siRNA) delivery. Xu et al. have imported Ad-PEG to a star-shaped cationic polymer consisting of poly(2-dimethyl amino)-ethyl methacrylate (pDMAEMA), grafted βCDs, and synthesized a supramolecular pseudoblock polycation (pDMAEMA-SS-CD@Ad-PEG). A nanoparticle is formed by conjugation of p53 plasmid DNA to the polycation and then coated with PEG for serum stability. The Ad-PEG coating reduces the polymer toxicity and improves the gene transfection efficacy. The nanoparticles enable efficient delivery of p53 plasmid into the tumor subsequently inhibiting tumor growth in mice.[12] Specific modification by means of targeting peptide or biodegradable linkage can also be implemented on the Ad-PEG chain to obtain additional functions.[13−15] Introduction of different cationic βCD derivatives to adamantane-coated dendrimers or different cationic adamantyl-end derivatives to βCD-containing polymers can modulate the surface charge.[16,17]

The βCD cavities can incorporate other guest moieties with an adamantyl end. Different species of the adamantyl-end moieties can be simultaneously included in a single nanoparticle. Poly(amido amine) dendrimer (PAMAM) with four or eight adamantyl ends (Ad-PAMAM) and Ad-PEG have been studied by Tseng's group in the development of a series of PEI−βCD based nanoparticles (Figure 2B).[18] Controlled self-assembly of the supramolecular nanoparticles is achieved by adjusting the molar ratio of PEI−βCD, Ad-PEG, and Ad-PAMAM. This self-assembly approach is termed the "bricks and mortar" strategy (Figure 2C) in which Ad-PEG and Ad-PAMAM with the guest adamantyl groups serve as the bricks while the PEI−βCD polymer bearing the host functionality serves as the mortar holding together the Ad-PEG and Ad-PAMAM. The conformational flexibility of the polymer compensates for the irregular size and shape of the guest moieties, thereby allowing convenient and efficient size control of the supramolecular nanoparticle during self-assembly. The Ad-PEG carrying the targeting peptides can be a complementary or alternative "brick" candidate in nanoparticle assembly in order to introduce targeting characteristics to the delivery system.[13,15,19,20]

Specific hydrophobic drugs are suitable dwellers in the βCD cavities, for instance, some anticancer agents (doxorubicin, camptothecin, paclitaxel, fluorouracil) and anti-inflammatory drugs (indomethacin, dexamethasone, ibuprofen), due to the ability to serve as guest molecules for βCDs.[21−25] Doxorubicin can be directly encapsulated onto the βCDs conjugated to quantum dots (CdSe/ZnSe QDs) and subsequently delivered by the nanoparticles.[22] The encapsulated doxorubicin released from endosome, in combination with cooperatively delivered mdr1 siRNA by the same vehicles, shows enhanced toxicity. The mdr1 siRNA silences the MDR1 mRNA responsible for multidrug resistance in cancer cells. Therefore, doxorubicin released intracellularly bypasses drug efflux mediated by a membrane transporter protein, P-glycoprotein (P-gp). A similar approach has been applied to polymer-grafted βCDs.[26] A triblock copolymer is modified to have one end with folic acid for targeting and the other end with βCD for guest recognition. Addition of hydrophobic doxorubicin induces the host−guest interaction with βCD and ensuing self-assembly of the drug-encapsulated copolymer. The center of the nanoparticle contains the βCD@doxorubicin structure and folic acid shelled by a hydrophilic coating derived from a block of the copolymer. This is usually considered as drug encapsulation into nanoparticles in the "core−shell" system. The typical core−shell nanoparticle system[25−27] has a hydrophobic core carrying insoluble drugs with high loading capacity whereas the hydrophilic shell provides a buffer or protection against the external environment (Figure 3). Many core−shell nanoparticles consist of amphiphilic block copolymers, and the shell can also be surface modified with functional groups.

Only a limited number of drug species is available for direct inclusion into the βCD. One solution is to use adamantyl decoration on the cargo drugs to produce the βCD@adamantane interaction for linkage between the drug and βCD-containing carrier. Adamantyl conjugation to the drug produces a prodrug and eliminates molecular steric requirements endurable by the βCD cavity. We have synthesized adamantyl-conjugated doxorubicin and paclitaxel.[28−30] Prodrugs self-assemble with βCDs cross-linked by the cationic PEI polymer, and the supramolecular nanoparticles are prepared in the presence of plasmid DNA or siRNA. The noncovalent linkage driven by the host−guest interaction leads to sustained drug release. By using a similar method, Zhang et al. have synthesized a supramolecular microcapsule by incorporating both adamantyl prodrug and adamantyl polymer into multilayer polymeric particles bearing βCDs.[31] The shell on the inorganic $CaCO_3$ particles is formed by self-assembly between polyaldehyde dextran-*graft*-adamantane (Ad-PAD) and carboxymethyl dextran-*graft*-β-cyclodextrin (CMD-βCD). By adopting the "bricks and mortar" strategy, adamantyl prodrugs with Ad-PAD are included in the βCDs via the host−guest interaction. Notably, adamantane and doxorubicin constituting prodrugs are linked by a hydrazone bond, and the prodrugs delivered to tumor cells release doxorubicin via acid cleavage of the hydrazone bond.

Another common host−guest interaction in delivery applications relies on the introduction of hydrophobic polymers such as poly(β-benzyl L-aspartate) (PBLA) and poly(*N*-isopropylacrylamide) (PNIPAA).[25,32,33] The PBLA chain polymers provide serial benzyl groups and contribute to self-assembly with the multiple recognition sites of βCD pendants on the PEI or PEG block polymers [PEI-*b*-(PEI−βCD) or PEG-*b*-(PEG−βCD)].[33] Complexation of hydrophobic PBLA and hydrophilic PEI-*b*-βCD produces core−shell nanoparticles. The PEI shell provides several advantages in the delivery system such as protection of the internal contents from the environment, increased solubility

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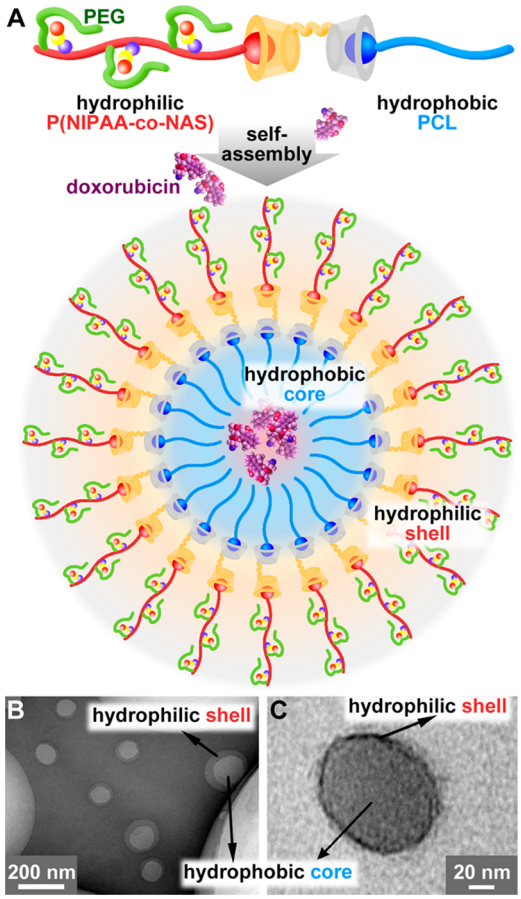

Figure 3. (A) Amphiphilic host–guest block copolymer encapsulating hydrophobic drugs in the hydrophobic core to form typical core–shell nanoparticles. (B, C) Electron microscopy images showing the hydrophobic core and hydrophilic shell of some core–shell nanoparticles. Reproduced from refs 27 and 33. Copyright 2010 American Chemical Society.

of the nanoparticles, and possible utility as gene vectors to condense plasmid DNA or siRNA. The PBLA and complementary βCDs on the PEI block polymer comprise a hydrophobic core thus creating a chamber to embed hydrophobic molecules (Figure 3C).

2.2. Major Host–Guest Designs Using α-Cyclodextrins

One of the popular and useful guest molecules for the αCD cavity is azobenzene. The two isomers of azobenzene with the *trans* and *cis* forms can be reversibly switched to each other upon photoirradiation (Figure 4).[34] Driven by hydrophobic effects and van der Waals interactions, *trans*-azobenzene can be recognized by αCD. When *trans*-azobenzene is transformed to *cis*-azobenzene under ultraviolet stimulation (wavelength $\lambda \approx$ 365 nm), αCD is no longer able to include the bulky *cis* form because of the mismatch between the host and guest. Interestingly, *cis*-azobenzene can transform into the *trans*-form simply with visible light exposure ($\lambda \approx$ 435 nm). Therefore, αCD and azobenzene constitute a good linkage in the delivery system where photostimulus induces the host–guest assembly and disassembly.[35]

Another αCD-based supramolecular architecture, pseudopolyrotaxane, is frequently employed in the nanoparticle design of delivery systems. In the pseudopolyrotaxane structure, cyclic αCDs are threaded onto a PEG chain axis and capped with a biodegradable bulky end moiety (Figure 4C). Inclusion of αCDs provides abundant hydroxyls as a supplement to the two functional groups at the terminal ends of the PEG. The hydroxyls act as functional groups for incorporation of targeting units, drugs, or nucleic acids. For instance, doxorubicin can be conjugated to αCD via the hydrolyzable ester linkage in the pseudopolyrotaxane capped by L-tyrosine ends.[36] Hydrolysis releases doxorubicin from the pseudopolyrotaxane in a sustained manner, and furthermore, cellular uptake of the doxorubicin-bearing polymer conjugates can be facilitated when a cell-penetrating protamine peptide is attached to the PEG end.

3. FUNCTIONAL, MORE FUNCTIONAL, MULTIFUNCTIONAL

Practical delivery systems need to possess extra functions to overcome the hurdles along the delivery route, and host–guest interactions facilitate the self-assembly of functional groups or co-operative delivery.

3.1. Inside the Nanoparticle: Size Control and Biodegradability

Physical properties like the nanoparticle size are of great importance to the biodistribution and acceptance of a delivery system.[37] With the inclusion system composed of βCD and adamantane, size-controllable synthesis of supramolecular nanoparticles can be achieved by Tseng's "bricks and mortar" method.[18] Another viable strategy is to replace the loose hydrogen bond with a host–guest interaction.[38] Mixing polyacids such as poly(acrylic acid) (PAA) and Lewis polybases such as PEG creates interacting complexes in an aqueous

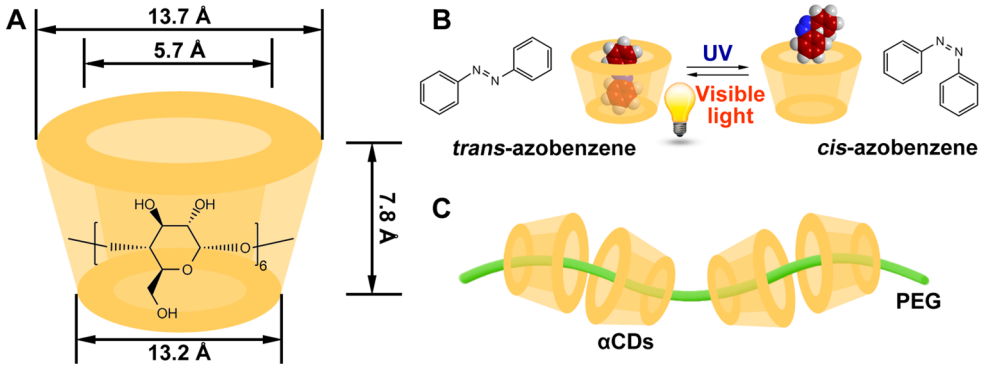

Figure 4. (A) Structure of αCD, (B) interaction of azobenzene and αCD enabling photoregulation based on azobenzene isomerization, and (C) αCDs threaded onto a PEG chain comprising the pseudopolyrotaxane architecture.

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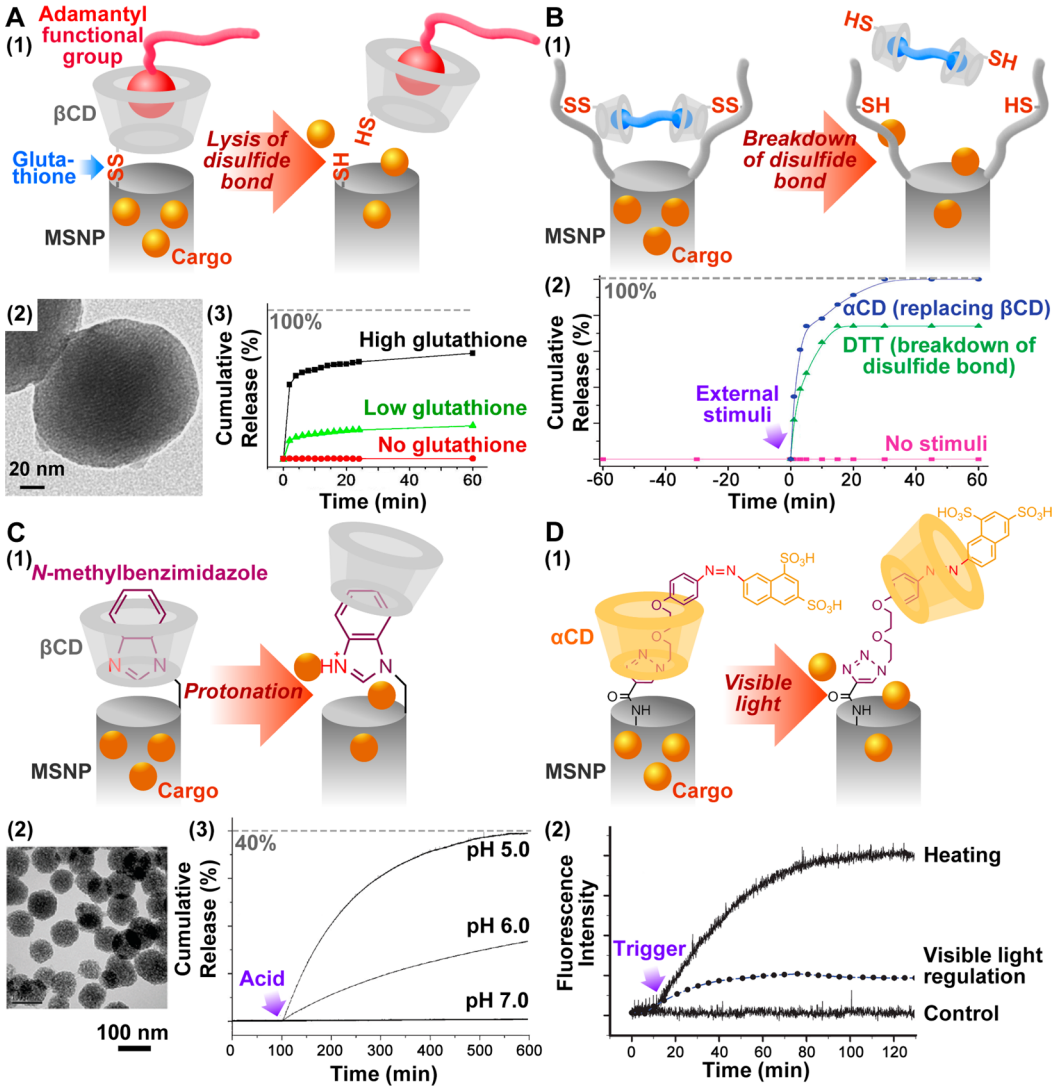

Figure 5. Gating structures of MSNP and cargo release kinetics: (A) lysis of disulfide bonds, breaking down the linkage between *β*CD and MSNP,[44] (B) breakdown of disulfide bonds resulting in cleavage of the cross-linked gating structure,[43] (C) protonation of *N*-methylbenzimidazole leading to *β*CD dissociation,[46] and (D) visible light stimulation causing *α*CD to form inclusions by *trans*-azobenzene after isomerization.[48] Adapted with permission, copyright 2009, 2010, and 2013 American Chemical Society and copyright 2012 Wiley.

solution held together by hydrogen bonds. The imported *β*CDs induce host−guest interaction with the PEG chain, which partially replaces the original hydrogen bond and reduces the size of the nanoparticle to facilitate delivery.

The toxicity of the delivery system depends on not only the nanoparticle size but also debris released during gradual decomposition after delivery. Hence, biodegradability is important. In addition to the reduced strength in the CD-based host−guest interaction compared with covalent bonds, introduction of hydrolyzable connections enhances intracellular degradation and clearance. However, high degradability may be worse since the nanoparticle will break down during delivery due to active hydrolysis. Therefore, the hydrolyzable linkage in nanoparticles should respond to environmental changes, especially pH or temperature. For example, cholesterols are modified by copolymer poly(vinyl alcohol)-grafted-PEG (PVA-*g*-PEG) via a pH-sensitive and hydrolyzable acetal linkage to form the host−guest interactions with cationic *β*CD derivatives. Controlled hydrolysis is accomplished by breaking down the acetal linkage in the acidic environment. The self-assembled nanoparticles carrying siRNA exhibit acid-triggered destabilization, low toxicity, and high transfection efficacy.[39]

3.2. Responding to Stimuli: Controlled Release

The extreme conditions in a tumor microenvironment such as elevated temperature and acidic pH due to hypermetabolism are well-known. There is increasing evidence suggesting the necessity of developing thermo- or pH-sensitive applications. Thermosensitivity in nanoparticles is usually related to the transition temperature, for instance, lower critical solution temperature (LCST). PNIPAA polymers have been incorporated into *β*CDs to interact with the adamantane-modified core in order to acquire thermosensitive characteristics.[40] Although PNIPAA shows a dramatic and reversible phase transition behavior in water with LCST at 32 °C, conjugation and inclusion in the supramolecular nanoparticle shifts the LCST to around 37 °C. When the temperature is slightly above the body temperature, the PNIPAA-containing host−guest pesudoblock copolymer changes from a clear solution with low viscosity to a stable hydrogel (self-assembly host/guest ratio of 1:1). The thermoreversible sol−gel transition allows the nanoparticle to undergo

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temperature-triggered gelation and release the delivered agents in a controlled manner. A similar sol−gel transition also occurs in the αCD-induced interaction with the guest copolymer of poly(L-glutamic acid) (PLGA) and PEG at approximately 55 °C.[41] The transition temperature is too high for delivery to tumors but may be suitable for subcutaneous release triggered by temperature. The pH sensitivity feature is available when the delivery system contains an acid-responsive linkage, which is usually located in the guest compartment.[27,39,42] A pH change can alter the sol−gel transition course originating from the host−guest interaction between the αCDs and PLGA-*b*-PEG copolymers.[41]

The use of disulfide bonds (-SS-) in nanoparticles boosts the redox sensitivity thus allowing the delivery system to detach some components under specific circumstances like endosomal lysis. Recently, we have synthesized a redox-responsive supramolecular nanoparticle self-assembled by peptide-modified PEI−βCD and adamantyl end PEG chain. In the guest groups presented as the polymer shell, adamantane and PEG are connected by the disulfide linkage (Ad-SS-PEG).[14] PEGlytion effectively improves the serum stability but decreases the transfection capability of polycations due to the reduction in the charge-mediated cellular uptake. Moreover, the neutralized shield hampers endosomal escape as a result of the reduced interaction between the polycation segments and endosomal membrane. Introduction of the redox-responsive Ad-SS-PEG structure can overcome the PEG dilemma by sensitive breakdown of the disulfide bond and subsequent detachment of PEG during endosomal lysis. The PEG-detachable delivery system boasts enhanced gene transfection efficiency as expected. The disulfide linkage can also be applied to the host part for redox-sensitive biodegradability when the shielding layers are connected to the nanoparticle core, for example, mesoporous silica nanoparticle (MSNP) and magnetic nanoparticle (MNP).[21,43−45] MSNP widely used in drug loading requires better control to release the contained species. Gating is required to block the drugs from release during delivery and to free the confined drugs to the destination. Linked with βCDs via disulfide bonds, MSNP can be shielded with adamantyl poly(aspartic acid) containing the targeting peptide (Arg-Gly-Asp motif, RGD) and matrix metalloproteinase (MMP) sensitive peptide (Pro-Leu-Gly-Val-Arg, PLGVR).[44] Triggered by the tumor, the nanoparticles undergo cleavage of the PLGVR peptide by MMP, followed by removal of gating βCDs by lysis of the disulfide bonds and release of the encapsulated anticancer drugs (Figure 5A). Similarly, polymers bearing βCDs linked by disulfide bonds (PNIPAA-SS-βCD) can be grafted to MSNP, and two βCDs cross-link with a diazo linker via the host−guest interaction to form the gating architecture. The trapped molecules are released from the hybrid MSNP materials by cleavage of the disulfide bonds in the cross-linked structure (PNIPAA-SS-CD@diazo-linker@CD-SS-PNIPAA) and loosening of the polymeric network on the outer surface of the MSNP (Figure 5B). Furthermore, environmental αCDs can compete against βCDs for the diazo linker to break the gating, because αCD favors the formation of inclusion complexes with the azobenzene group over βCD.[43] Addition of competitive molecules, particularly αCD host in this case, is a release strategy with external control. In addition, pH-sensitive interactions between *N*-methylbenzimidazole and βCD can help build the gating structure on MSNP when protonation at low pH causes dissociation of βCD caps and release of cargo molecules (Figure 5C).[45,46] Such protonation and deprotonation processes with pH sensitivity also allow for benzimidazole-stalk controlled release in core−shell nanoparticles.[47]

Controlled release as a result of external stimuli such as light, magnetic field, voltage, or molecular competition may be adopted in delivery. Photosensitivity is usually accomplished by host−guest interactions between αCD and azobenzene based on the switchable azobenzene isomer under light regulation.[34] Photoresponsive delivery systems can achieve spatial and temporal release of the therapeutic cargo by remote activation. A recent study applies the αCD@azobenzene interaction together with [2]rotaxanes to enable photoresponsive gating on MSNP.[48] In particular, a linear azobenzene axle with the αCD ring is end-capped by a naphthalene stopper. The bulky stopper unit has two sulfonic groups to enhance the aqueous solubility. Upon exposure to ultraviolet or visible light, azobenzene isomerization causes the corresponding αCD movement to close or open the nanopores (Figure 5D). Besides, the interaction between βCD and azobenzene exhibits a light-regulated behavior.[49] The on/off photoresponsive effect allows remote control of drug storage and release. With regard to magnetically controlled release, the common strategy is to include a MNP core in the delivery system or to use adamantyl MNPs as guest structures according to the "bricks and mortar" method.[21,50] Recent work reveals that the voltage regulates the assembly/disassembly process in some host−guest delivery systems. Yuan et al. have developed a core−shell pseudo-block polymer composed of polystyrene with βCD end decoration and PEG with an uncharged ferrocene end.[51] The uncharged ferrocene binds to the βCD cavity strongly, whereas the charged ferrocene dissociates rapidly from βCD. The two species of end-decorated homopolymers self-assemble to form the shell protecting the encapsulated cargo and dissemble to release the load when a positive voltage is applied. Therefore, controlled release by applying external stimuli is a promising therapeutic approach although current techniques have only been demonstrated *in vitro* or subcutaneously rather than in tumors inside the body.

3.3. Finding a Destination: Targeted Delivery

Targeted delivery boasts higher specificity to the desired destinations and enhanced delivery efficacy. The common method to obtain targeting features on nanoparticles is modification with targeting moieties. It may be facilitated by incorporation of guest components into the supramolecular delivery system. Targeting groups such as RGD peptides have been conjugated covalently to Ad-PEG, and the modified Ad-PEGs are assembled to βCD-containing nanoparticles, supplemented by the unmodified Ad-PEGs for surface PEGlytion.[13,15] The RGD sequence improves the cellular uptake efficacy because RGD-binding $\alpha_v\beta_3$ integrins are overexpressed on the surface of tumor cells. In a more complicated design, the guest part consists of a phenyl group for the host−guest interaction, a PEG-*b*-PNIPAA copolymer chain for biocompatibility and thermosensitivity, and an RGD peptide end for targeting. Inclusion of the guest into the nanoparticle enables targeted delivery to $\alpha_v\beta_3$ integrins.[27] Similarly, hypermetabolism of iron by cancer cells may lead to overexpression of transferrin receptors, making transferrin a targeting molecule. In the advanced case, the synthetic nanoparticle contains a βCD-based polymer, transferrin-conjugated Ad-PEG guests to target cancer cells, Ad-PEG guests for protection and biocompatibility, and siRNA to reduce the expression of ribonucleotide reductase subunit M2 (RRM2). RRM2, an established anticancer target, can be attenuated by

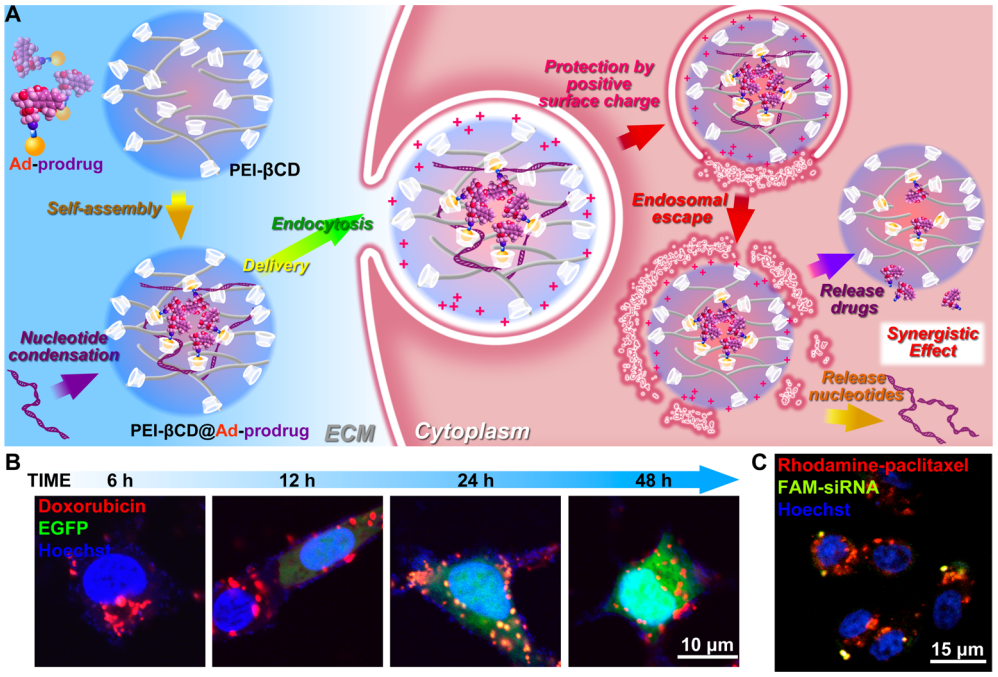

Figure 6. (A) Self-assembled host–guest complexes PEI–βCD@Ad-prodrug with nucleotide condensation ability for co-delivery of drugs and genes into the cytoplasm with polycations assisting the nanoparticles to escape from the endosome and achieving synergistic effects after release of drugs and nucleotides. (B) Time-dependent co-delivery achieving delivery of doxorubicin prodrug and transfection of EGFP plasmid DNA in human ovarian carcinoma SCOV-3 cells,[28] and (C) tracking of co-delivered rhodamine-labeled paclitaxel and FAM-labeled siRNA in SCOV-3 cells[29] via confocal microscopy. Adapted with permission, copyright 2012 Elsevier.

RNA interference (RNAi) after effective delivery of the RRM2 siRNA by the targeted nanoparticles. Human phase I clinical trials involving the targeted siRNA delivery system show reduction of RRM2 mRNA and proteins by RNAi from systemically administered siRNA.[15] It is noteworthy that the host can also carry targeting groups and help deliver cargo to the target.[14,26]

3.4. Multifunctional Systems

Owing to the convenience of guest inclusion in the delivery system, additional functions can be included in the hybrid nanoparticles by modification of the guest groups. Delivery systems have been designed to possess dual- or multiresponsive properties,[41,42] sometimes in combination with the targeted approach, particle size control, or enhanced biodegradability.[14,19,27,39,44,50]

4. CO-DELIVERY

Nanoparticles are being studied for multiple deliveries. By taking advantage of the host–guest self-assembly, we have developed a system capable of both gene transfection and drug delivery termed as co-delivery mediated by host–guest nanoparticles (Figure 6A). Co-delivery of gene segments and drugs enhances gene expression or achieves synergistic/combined effects of chemotherapies and gene therapies.[52] We have synthesized an adamantyl-ended prodrug of anticancer therapeutic (Ad-prodrug) that can release the chemotherapeutic drug in a sustained manner. The prodrugs are imported into the supramolecular materials via adamantane interaction with βCDs located in a cross-linking PEI network to form a prodrug-encapsulated PEI–βCD hybrid polymer in an aqueous solution. The PEI–βCD@Ad-prodrug complexes possessing cationic properties are able to condense anionic nucleotides by electronic interactions. When bonded with plasmid DNA or siRNA in the nanosystem, the supramolecule transforms into a sphere-like nanoparticle with a slightly reduced size, which can traverse along the vessels *in vivo*, but the surface charge remains positive. The corona of the cationic charges on the nanoparticles allows establishment of the disperse system and prevents aggregation.

By employing this prodrug containing nanoparticle, we have functionalized the delivery system with loaded prodrug and a condensed therapeutic gene, which have synergistic effects on tumor cells (Figure 6B,C). Adamantyl–paclitaxel prodrugs encapsulated on the core of the supramolecular micelle are able to gradually release the free anticancer therapeutics. Meanwhile, the micelles carry a survivin small hairpin RNA (shRNA)-encoding plasmid to silence survivin expression via RNAi. The cationic charges protect survivin shRNA from endolysis during nanoparticle endocytosis and assist the shRNA to escape from the endosome. The internalized nanoparticles release the co-delivered paclitaxel and survivin shRNA, and these show a synergistic effect on enhancing apoptosis and inhibiting tumor growth.[29] In another case, we have co-delivered doxorubicin and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-encoding plasmid using a similar vector. Co-treatment of TRAIL transgene therapy leads to doxorubicin with significantly enhanced apoptosis, thus limiting cancer proliferation and tumor growth *in vivo*.[28,30] Similarly, a QD-based host–guest nanoparticle developed by Mao's group can perform *in vitro* co-delivery of doxorubicin and mdr1 siRNA.[22]

5. CHALLENGES AND FUTURE PERSPECTIVES

Functional host–guest nanoparticles are designed for practical applications such as *in vivo* drug or gene delivery, but limited clinical therapeutics have been reported.[15,53] Functional delivery

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for systemic administration encounters circulation problems, anatomical and cellular barriers, systemic toxicity, and especially for functional nanoparticles, attenuation of designed functions like tumor targeting.[54] More translational work is required to overcome these difficulties. The replaceable guests, possibly with a highly applicable host molecule like liposomes,[55] allow new designs with controllable size suitable for circulation dynamics or with altered surface charges to promote permeability of the blood–brain barrier and cellular endocytosis. Novel targeting moieties screened by phage display or molecular mimicking technologies may yield higher specificity in targeted delivery. Future directions include (1) multifunctional design with controlled response ability, even for single-dose injection, to optimize release triggered by the tumor microenvironment and to reduce systemic toxicity to enhance patients' tolerance, (2) development of convenient dose administration with better biocompatibility, such as injectable supramolecular hydrogel based on molecular recognition between the CDs and hydrophobic compounds, (3) incorporation of functional features in co-delivery to build a responsive system with synergistic effects, and (4) update of the switchable self-assembly compartments to enable efficient synthesis, lower cost, high specificity, and clinical potential. With better and more optimal schemes, multifunctional CD-based nanoparticles with co-delivery ability have immense potential in clinical applications.

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