Trends in Chemistry

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Review

Supramolecular *in situ* assemblage and its biological applications

Hengzhi Zhang (张珩之)[1,2], Shuangqi Song (宋双骐)[1,2], and Yu Liu (刘育)[1,*]

Water-soluble macrocycles, including cyclodextrins (CDs), cucurbit[n]urils (CB[n]s), sulfonated calixarenes (CAs), and pillararenes (PAs), can selectively bind organic anions or cations for *in situ* assemblage to construct supramolecular assemblies. These assemblies can also achieve spatially-confined luminescence or encapsulate drugs, demonstrating their wide applications in biology and medicine, as well as other fields. This review discusses biological applications of supramolecular *in situ* assemblies constructed by water-soluble macrocyclic derivatives, including macrocyclic hosts modified by polymers, functionalized guests, and macrocyclic hosts, and noncovalent supramolecular polymers. With further development of supramolecular *in situ* assemblies, intelligent systems with pH, light, and enzyme responsiveness can be widely integrated in macrocyclic cavities with spatially-confined luminescence for cell imaging, targeted drug delivery, and *in situ* drug formation.

Highlights

Supramolecular macrocyclic hosts such as cyclodextrins, cucurbit[n]uril, and calix[n]arenes can be covalently or noncovalently linked to active molecules to construct *in situ* supramolecular assemblies.

Stimuli-responsive nanofibers formed by *in situ* supramolecular assemblage can modulate cell morphology or induce cell apoptosis. Also, targeted imaging and therapy are realized utilizing the recognition between supramolecular assemblies and specific receptors or organelles.

Through the noncovalent polymerization of two- or three-arm guest molecules and macrocyclic hosts, the luminescence of guests is enhanced and red-shifted due to J-aggregation, which has high penetration to biological tissues, making it suitable for bioimaging and photodynamic therapy (PDT).

In situ supramolecular assemblies

In situ supramolecular assemblies constructed by water-soluble macrocyclic compounds, with different hydrophobic cavities as hosts to recognize positively-charged, neutral, or negatively-charged guests through host–guest interaction, are widely used in the fields of cascade energy transfer, efficiency enhancement of chemicals [1–3], cell imaging, *in situ* diagnoses [4–9], targeted drug delivery, *in situ* drug formation [10–15], and other chemical or biological applications. Among them, **cyclodextrins (CD)** (see Glossary) can bind negatively charged or neutral fluorescent dyes and drugs to improve their water solubility [16,17]. In contrast, **cucurbit[n]urils (CB[n]s)** [18,19], **calixarenes (CAs)** [20,21], and **pillararenes (PAs)** [22,23] are formed by the condensation of glycoluril, p-tert-butylphenol derivatives, and hydroquinone derivatives with formaldehyde, respectively, and they all have a higher affinity for positively-charged guests due to the high negative potential on the carbonyl group of the glycoluril unit and the high π electron density on the benzene ring. Among them, CB[n] (n = 6,7) with a smaller cavity can bond with guest molecules in a 1:1 stoichiometric ratio, while CB[8] with a larger cavity tends to bond with guest molecules in a 1:2 stoichiometric ratio. Because the benzene ring in CAs and PAs is easily modified singly or multiply, diverse functional groups can be modified to enhance their water solubility and achieve stimulus responsiveness [24].

This review mainly focuses on three areas of research regarding macrocyclic assemblies for biological applications:

(i) *In situ* supramolecular assemblies formed by polymers modified by four macrocyclic hosts and guest molecules, such as CD-modified hyaluronic acid (HA) or graphene oxide, which can bond with a variety of drug molecules and release them after being taken up by tumor cells. The further co-assembly of HA CD with magnetic nanoparticles can be induced by external magnetic fields to produce nanofibers in cells, thereby destroying the cell structure [25,26].

[1]College of Chemistry, State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, China
[2]These authors contributed equally to this work

*Correspondence:
yuliu@nankai.edu.cn (Y. Liu).

(ii) *In situ* supramolecular assemblies formed by macrocyclic hosts and modified guest molecules [27,28]. The guest molecules are modified on functional peptides or proteins. By bonding with the macrocycle, their aggregation morphology can be regulated, or the activity of functional proteins can be selectively turned on/off.

(iii) *In situ* supramolecular assemblies constructed by noncovalent polymerization. Macrocyclic compounds with larger cavities can bond two guest molecules head to tail, forming a 1:2 long chain or network supramolecular noncovalent polymer to promote photoluminescence properties and reveal effective triplet state to singlet state **Förster resonance energy transfer** (TS-FRET), thus attaining long-lived near-infrared (NIR) luminescent systems for cellular imaging [29] (Figure 1).

As compared with previous reviews, this review concentrates more on biocompatible water-soluble macrocycles like bioagent CDs, CAs, etc. By modifying with charged functional groups, these macrocycles can bind insoluble drugs and induce the luminescence of chromophores to improve their bioavailability and induce upconversed NIR excitation [30,31], promoting their applications in bioimaging [32,33] and synergistic therapy [34,35].

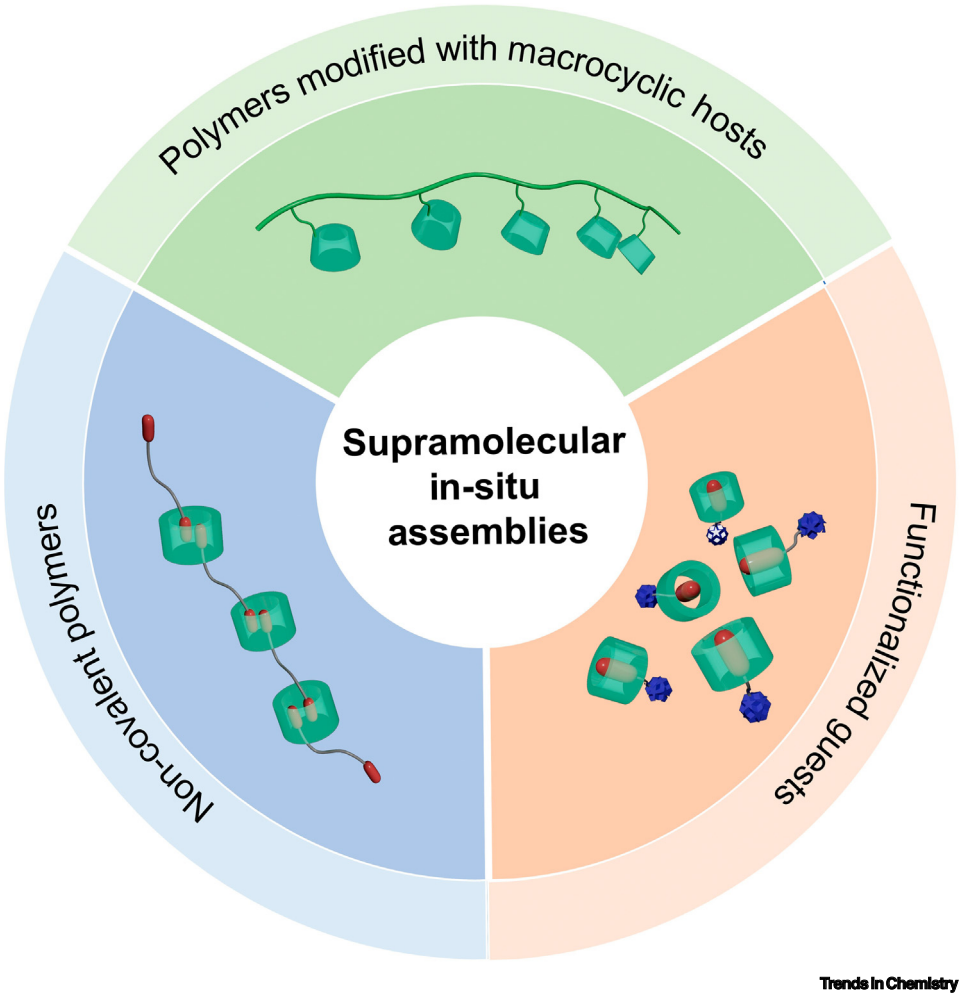

Figure 1. Schematic illustration of *in situ* supramolecular assemblies and structures of four common macrocycles.

Glossary

Calix[n]arenes (CA): cyclic molecules obtained by the condensation of para-t-butylphenol and formaldehyde, including calix[4]arenes, calix[6]arenes, and calix[8]arenes. The sulfonated calixarenes (SCA), which are obtained by sulfonating the phenyl rings on them, have high water solubility.

CD44 and CD168: two receptors that can bind to hyaluronic acid, which are overexpressed on the surface of tumor cells and involved in the adhesion and metastasis of tumor cells.

Cucurbit[n]uril (CB[n]): cucurbit[n]uril is formed by the condensation of glycoluril and formaldehyde. The carbonyl groups at the ends have a high negative potential and exhibit strong affinity towards positively-charged guests.

Cyclodextrins (CD): cyclic molecules formed by α-d-pyran glucose units linked by 1,4-glycosidic bonds. α-, β-, and γ-cyclodextrins contain 6, 7, and 8 glucose units, respectively.

Förster resonance energy transfer: the energy transfer process between excited-state and ground-state molecules, through dipole–dipole interaction, nonradiatively transfers the energy of the excited-state donor (excited singlet, SS-FRET, or excited triplet state, TS-FRET) to the ground-state acceptor at a distance of 1–10 nm.

Immunogenic cell death (ICD): when tumor cells die in response to external stimuli, they change from non-immunogenic to immunogenic, thereby mediating the generation of antitumor immune responses in the body.

Photodynamic therapy (PDT): utilizing the reactive oxygen species generated by photosensitizers under light excitation to induce oxidative stress, leading to lipid peroxidation of cell membranes, protein denaturation, DNA damage, etc., and ultimately inducing apoptosis or necrosis of cells.

Pillar[n]arenes (PA): macrocyclic compounds obtained through condensation reactions between p-dimethoxybenzene derivatives and formaldehyde. The benzene units are connected by methylene bridges and can freely rotate.

In situ supramolecular assemblies formed by polymers modified with macrocyclic hosts

HA is a linear polysaccharide biological agent containing alternating β-1,4-d-gluconic acid and β-1,3-*N*-acetyl-d-acetylglucosamine units (Figure 2A) [36]. The abundant amino and hydroxyl groups on it can bind to receptors overexpressed on the surface of tumor cells (such as **CD44** [37] and **CD168**), which also allow it to be easily modified by macrocyclic derivatives [38,39]. For example, through condensation between the carboxyl group in HA and mono-ethylenediamine-modified CD, HA modified with permethyl-β-CD can bond with porphyrin units in the prodrug TPP-CA4 to construct prodrug nanoparticles (Figure 2B) [40]. The nanoparticles can be recognized by the CD44 receptor on the surface of tumor cells and enter the cells. Under the irradiation of 660 nm light, the nanoparticles disintegrate, causing the release of the photodynamic agent tetraphenylporphyrin and tubulin binder CA4. Tetraphenylporphyrin can produce singlet oxygen species under red light irradiation and induce oxidative damage to tumor cells, while CA4 can inhibit tubulin polymerization and thus block the mitosis of tumor cells, thereby achieving a synergistic antitumor effect. Taking advantage of the bonding ability of the β-CD cavity to ferrocene, a drug-loaded nanoparticle co-assembled with HA-modified cyclodextrin (HACD) and ferrocene-modified camptothecin was constructed [41]. The high concentration of H_2O_2 in tumor cells oxidized the ferrocene unit to a positive ion, weakening its bonding with HACD. Subsequently, the disulfide bond between ferrocene and camptothecin units was also broken by H_2O_2, resulting in the rapid release of camptothecin, achieving the effect of inhibiting tumor cell growth. In addition, the modification of permethyl-β-CD and the mitochondrial targeting group triphenylphosphonium to HA made it dual targeting [42]. The primary assembly formed by camptothecin-CD and porphyrin undergoes secondary assembly with the dual targeting HA, while the prodrug nanoparticles are formed through the recognition of the adamantane on the porphyrin and the CD cavity. After being ingested by tumor cells, the nanoparticles can be targeted to the mitochondria, where the disulfide bonds are cleaved under the action of overexpressed glutathione, releasing camptothecin while porphyrin can produce singlet oxygen species under light, leading to mitochondrial dysfunction and ultimately inducing cell apoptosis.

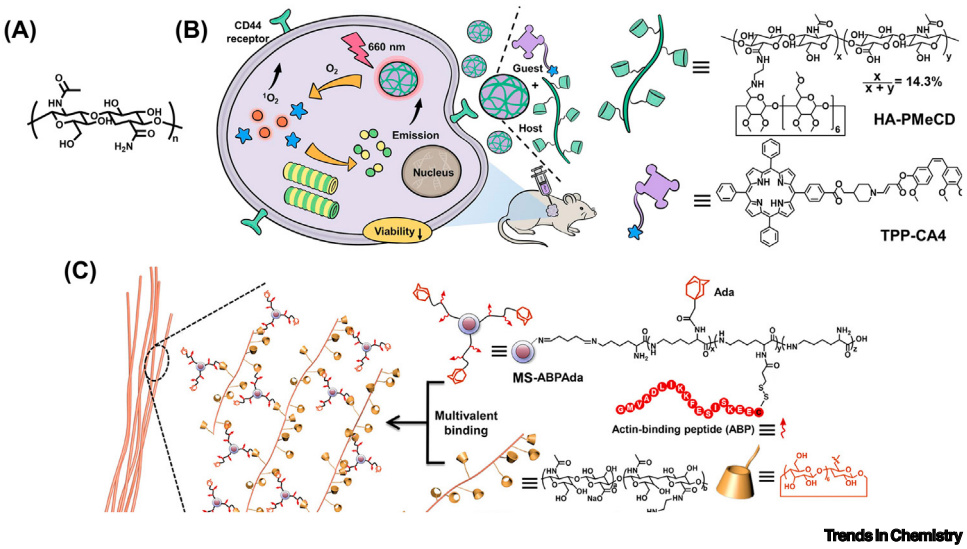

Figure 2. Examples of *in situ* supramolecular assemblies constructed by hyaluronic acid (HA)-modified cyclodextrin. (A) Structure of HA. (B) Schematic illustration of HA-modified permethyl-β-cyclodextrin for tumor synergistic therapy. (C) Schematic illustration of magnetic supramolecular *in situ* assembly. B, readapted from [40], with permission from American Chemical Society. C, readapted from [43], with permission from American Chemical Society.

HACD can also be co-assembled with magnetic nanoparticles to form nanofibers with directional growth in cells through external magnetic fields. For example, magnetic silica nanoparticles modified by actin-binding peptide and adamantane groups can co-assemble with HACD (Figure 2C) [43]. The assembly can form obvious nanofibers under an external magnetic field of 3 mT. When incubated with dental pulp stem cells in a magnetic field, the nanofibers in the cells can drive the extension of the cell tip and cell polarization. Similarly, polylysine modified with actin-binding peptide and adamantane grafted on the surface of magnetic Fe_3O_4 particles can form a multivalent assembly with HACD, targeting the actin skeleton in cancer cells [44]. Under the action of a low-frequency alternating magnetic field (200 Hz), the nanofibers in the cells induce actin rupture, causing cell cycle arrest and promoting cell apoptosis.

Similar to HA, chitosan (CS) is another linear polysaccharide in which the exposed amino groups usually bind to protons [45], showing positive charge and thus possessing high affinity towards negatively charged CD. For example, the supramolecular cascade reactor constructed by CS, sulfobutylether-β-CD, ferrous ions, glucose oxidase, and vinyl monomer polyethylene glycol diacrylate (PEGDA) can be used for *in situ* drug formation of diabetic wounds (Figure 3A) [46]. Specifically, glucose oxidase catalyzes the oxidation of glucose to produce H_2O_2, which is further cleaved to produce hydroxyl radicals •OH, which not only have a strong antibacterial effect but also trigger the polymerization of vinyl monomers to achieve *in situ* coagulation and cover wounds, thereby inhibiting infection and promoting healing. The hydrogel formed by CS, silver

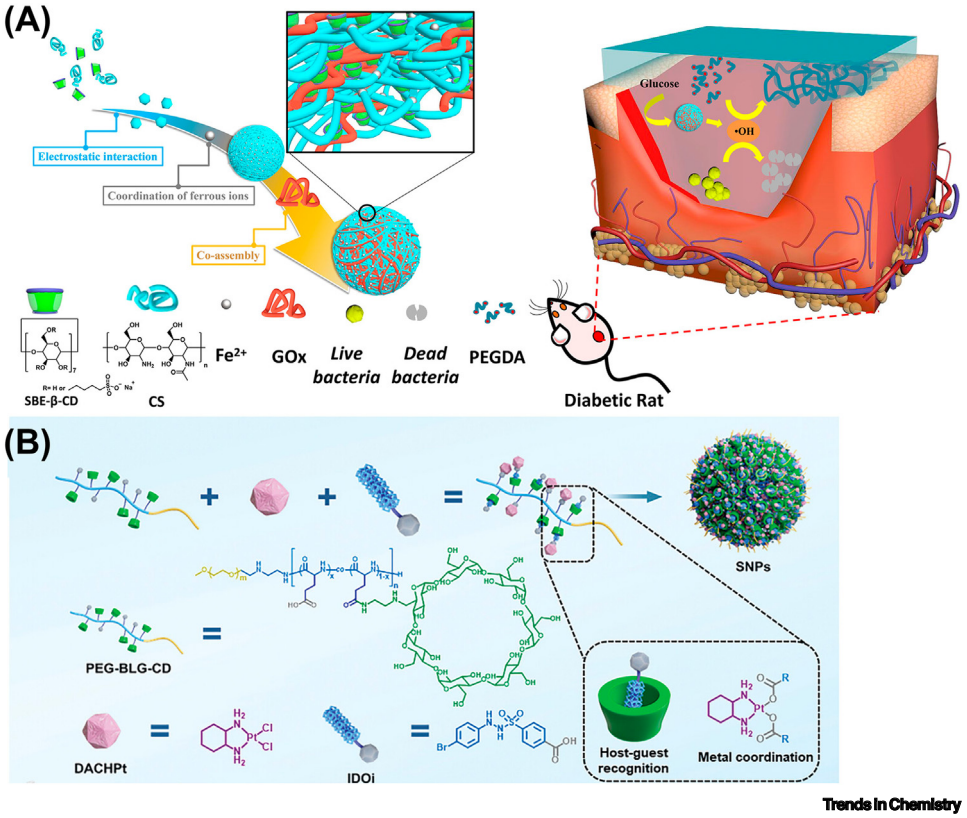

Figure 3. Examples of *in situ* supramolecular assemblies constructed by other cyclodextrin (CD)-modified polymers. (A) Schematic illustration of *in situ* supramolecular cascade reaction for diabetic wound healing. Readapted from [46], with permission from American Chemical Society. (B) Schematic illustration of *in situ* supramolecular assemblies constructed by β-CD-modified peptide copolymer. Readapted from [50], with permission from the authors.

ions, and sulfobutylether-β-CD can load doxorubicin in the cavity of sulfobutylether-β-CD [47]. Because the gel can coagulate again after injection, it can be injected to achieve *in vitro* drug delivery for bladder cancer. In addition, it can stably release doxorubicin over a long period, extending the dosing interval.

Furthermore, graphene and peptides/proteins can also be modified by macrocycles to participate in *in situ* assembly. Graphene oxide modified with CD can load magnetic nickel nanoparticles and doxorubicin, and further co-assemble with mitochondrial targeting peptide grafted HA, in which the cyclohexyl group on the mitochondrial targeting peptide can be encapsulated by the cavity of CD [48]. The drug carrier was taken up by tumor cells and targeted to mitochondria through the recognition of CD44 receptors. Doxorubicin was released from the assembly under the action of an external magnetic field, severely damaging mitochondria and cell nuclei. CD modified on graphene oxide can also bond with azobenzene units in polylysine, mitochondrial targeting peptides, azobenzene, and polyethylene glycol-modified transferrin to form an assembly targeting tumor cell mitochondria [49]. Due to the responsiveness of azobenzene to ultraviolet/visible light, ultraviolet/visible light can be used to regulate its assembly and disassembly in cells, thereby regulating the level of mitochondrial aggregation, while graphene oxide also has a photothermal effect under NIR light irradiation. It achieves a synergistic therapeutic effect of mitochondrial dysfunction and photothermal effect. The peptide copolymer carrier PEG-BLG-CD modified with CD can bind to IDO-1 inhibitor and platinum anticancer drug DACHPt, where DACHPt can induce **immunogenic cell death (ICD)** of tumor cells, thereby activating the antitumor immune response, while IDO-1 inhibitor can inhibit immune escape of tumor cells, ensuring long-term antitumor immune response (Figure 3B) [50]. In addition, HAS protein modified with sulfonated azocalixarene can load hydroxychloroquine and mitochondrial photosensitizer and release them in the hypoxic environment of tumor cells, synergistically leading to oxidative stress and mitochondrial damage. Therefore, we can achieve rapid development of *in situ* assemblies based on bioagent polysaccharide and we hope to promote their wider application in biology by combining with enzyme protein.

In situ supramolecular assemblies formed by functionalized guests and macrocyclic hosts

It is well known that permethylated-β-CD has a high bonding ability for photodynamic agent porphyrin [51], thus, a series of supramolecular *in situ* assembled **photodynamic therapy (PDT)** systems have been constructed based on functional porphyrin guests. For example, permethylated-β-CD modified with antimitotic peptide and porphyrin modified with cell-penetrating peptide can bond in a 2:1 stoichiometric ratio [52]. After entering tumor cells, the assembly can form spherical tubulin aggregates through peptide-tubulin recognition and produce singlet oxygen species under 660 nm light, synergistically inducing cell apoptosis and tumor ablation. Morpholine-modified permethylated-β-CD can form a 2:1 stoichiometric host–guest complex with sulfonated porphyrin and then undergo secondary assembly with CS modified by folic acid [53]. The assembly, which can recognize the overexpressed receptors on the surface of tumor cells through the morpholine and folic acid on the surface, produced reactive oxygen species (ROS) under light, which induced cell damage and promoted cell death. In addition, two photosensitizers, sulfonated aluminum phthalocyanine and adamantane-modified pyrene derivatives, can aggregate through ππ stacking (Figure 4A) [54]. The adamantane can undergo molecular recognition with CD modified by folic acid to form nanoparticles, which enter the cells through the uptake of folic acid by tumor cells. Subsequently, the sulfonated aluminum phthalocyanine migrates to the lysosomes, while the pyrene derivatives aggregate in the mitochondria. Under light, both photosensitizers can efficiently produce singlet oxygen species, and the sulfonated aluminum phthalocyanine can migrate from the lysosome to the cell nucleus under light, which can monitor the death of tumor cells in real time.

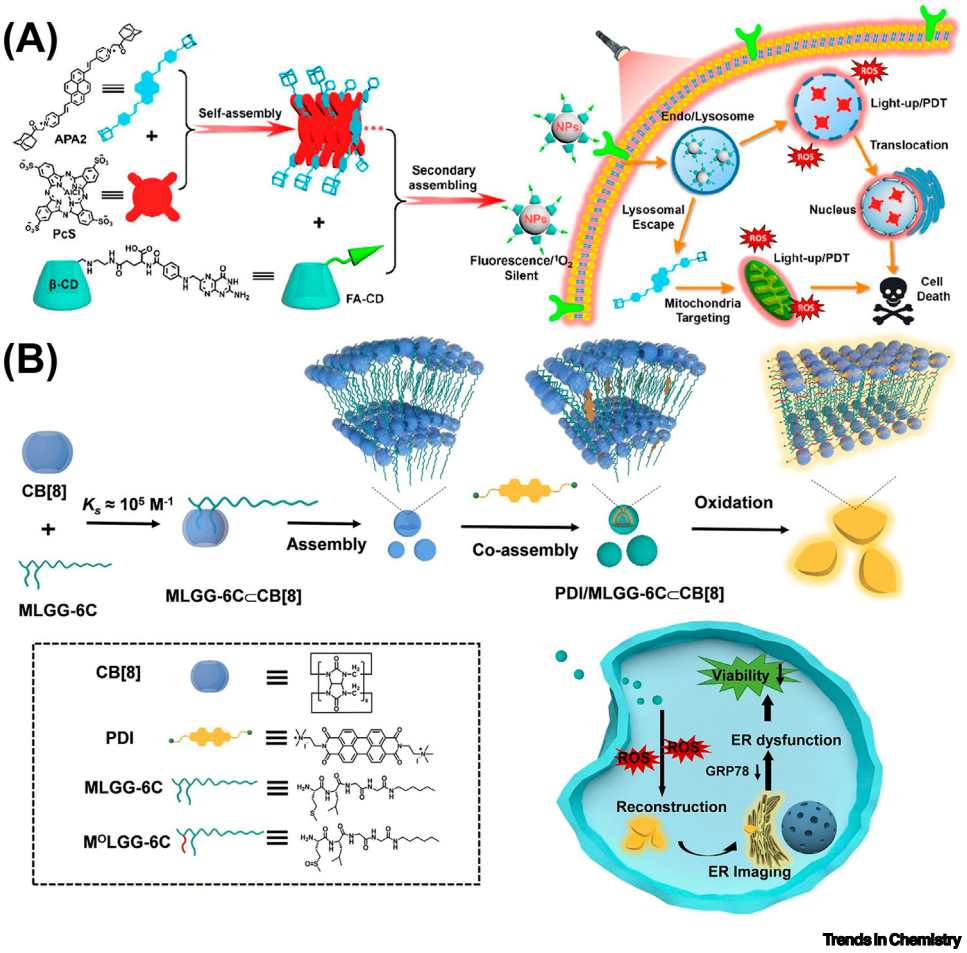

Figure 4. Examples of *in situ* supramolecular assemblies constructed by functional small molecules. (A) Schematic illustration of *in situ* supramolecular assembly for photodynamic therapy (PDT) treatment. (B) Schematic illustration of *in situ* supramolecular assembly based on CB[8] and peptides. A, readapted from [54], with permission from American Chemical Society. B, readapted from [56], with permission from the authors.

The macrocyclic hosts can also participate in supramolecular *in situ* assembly through host–guest interactions with other functionalized peptides. For example, the modification of adamantane on the tumor cell-targeting cyclic arginine–glycine–aspartate peptide can make it recognizable by β-CD modified with biothiol probe to form a host–guest complex, which can achieve efficient penetration into tumor cells [55]. At the same time, the probe shows reversible and rapid responsiveness to biolthiols, which can also monitor the biothiol concentration in tumor cells in real time. In addition, the side chain of the methionine-containing amphiphilic peptide MLGG-6C can bind to CB[8] with a bonding constant more than 10^5 M^{-1} to form an amphiphilic assembly (Figure 4B) [56]. Further addition of quaternary ammonium modified perylene diimide, which serve as competitive guests to CB[8], can co-assemble to form a multivalent supramolecular assembly. After the assembly enters the cancer cell, the high level of ROS in it oxidizes the methionine residue from a hydrophobic thioether to a hydrophilic sulfoxide or sulfone, which significantly reduces its bonding ability with CB[8], causing more quaternary ammonium modified perylene diimide to enter the CB[8] cavity, activating fluorescence emission and achieving endoplasmic reticulum imaging, interfering with endoplasmic reticulum function, and ultimately inducing cancer cell death.

Supramolecular *in situ* assemblies can also be constructed by modifying molecular recognition sites on natural or artificial polymers or simply doping with macrocycles [57–59]. HA can not only be modified by macrocyclic hosts, but also modify guest molecules to undergo molecular recognition with macrocyclic hosts. For example, bromophenylpyridinium-modified HA can bond with CB [8] in a 1:2 stoichiometric ratio, emitting room temperature phosphorescence at 500 nm through the confinement of the macrocycle, which can be used for targeted imaging of tumor cells [60]. Further modification of mitochondrial targeting peptides and bromophenylpyridinium on HA can result in it being taken up by tumor cells and entangling with mitochondria. Since CB[8] can bond with bromophenylpyridinium in a 1:2 stoichiometric ratio, incubation of these tumor cells with CB[8] can cause mitochondrial aggregation, thereby inducing cell apoptosis and completely inhibiting tumor cell growth *in vivo*. At the same time, the confinement of the macrocycle causes bromophenylpyridinium to emit room temperature phosphorescence for labeling. In addition, unmodified HA can also be assembled with host–guest complexes through secondary assemblage. For example, the NIR dye modified with the anticancer drug chlorambucil can be bonded with CB [7] in a 1:1 stoichiometric ratio and then assembled with HA in the secondary assemblage to obtain prodrug nanoparticles (Figure 5A) [61]. HA recognizes receptors overexpressed on the surface of tumor cells, allowing the nanoparticles to be internalized into the cells, and then the ester bond in the prodrug is decomposed under the action of butyrylcholinesterase, releasing nitrogen mustard and emitting NIR fluorescence synchronously.

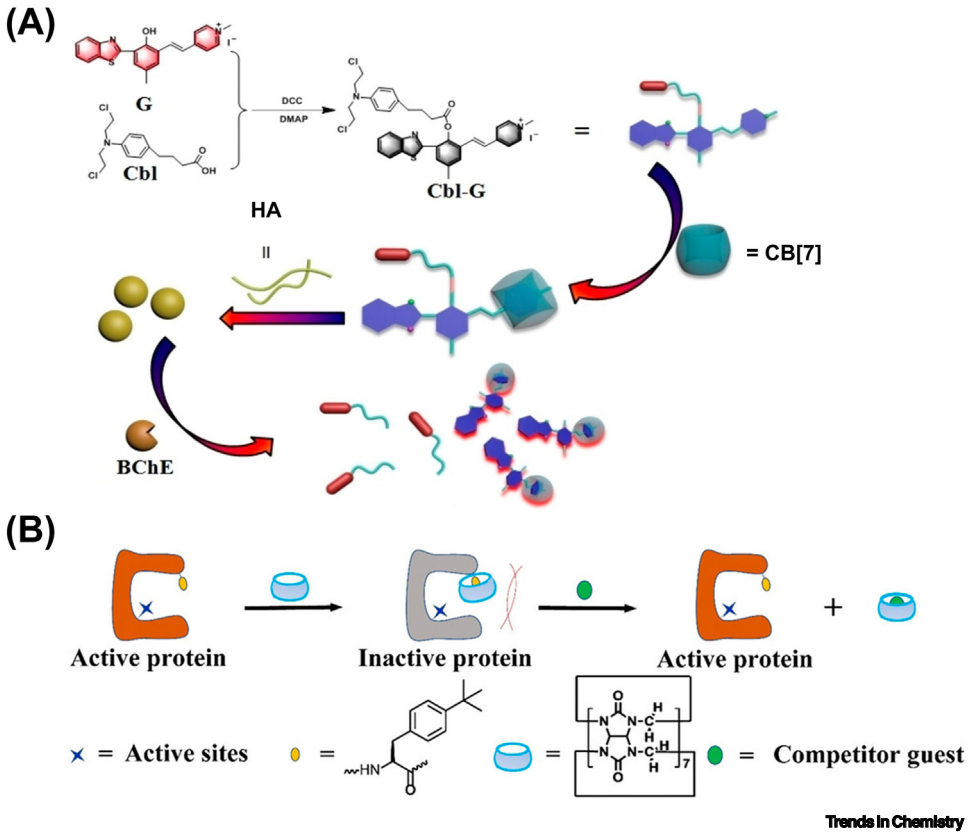

Figure 5. Examples of *in situ* supramolecular assemblies constructed by functional polymers. (A) Schematic illustration of dual functionalized hyaluronic acid (HA) co-assembled prodrug. (B) Schematic illustration of functional protein regulated by CB[7]. A, readapted from [61], with permission from Wiley-VCH. B, readapted from [65], with permission from Wiley-VCH.

Similarly, molecular recognition sites can be modified on peptides/proteins to form *in situ* assemblies [62]. For example, benzylimidazolium can be modified on antimitotic peptides [63]. Since antimitotic peptides can recognize tubulin, benzyl imidazole salts, which can recognize CB[8], are embedded on the surface of tubulin and bonded with CB[8] in a 2:1 stoichiometric ratio, resulting in crosslinking between tubules and inducing microtubule aggregation, leading to significant cell apoptosis and tumor ablation. However, adamantane modified on transferrin can be assembled with CD-modified ruthenium complexes to form nanoparticles, which enter tumor cells through transferrin receptors on the surface of tumor cells [64]. Under light, the ruthenium complex can produce ROS and destroy lysosomes. In addition, non-natural amino acids can be inserted into the surface of proteins through genetic coding and their side chains can be recognized by CB[7] (Figure 5B) [65]. When bonded with CB[7], the active sites inside the protein are inactivated due to steric hindrance. After adding competitive guests, CB[7] is removed from the protein and its activity can be restored, providing a simple and universal strategy for regulating protein activity. Amphiphilic phosphatidylcholine and phosphotyramine-modified β-CD can be assembled to form a nano-therapeutic system. Interestingly, chiral nanoparticles formed by d/l-arginine and carboxymethyl pillar[5] arenes have different binding abilities towards liposomes [66], with the binding ability of nanoparticles formed by d-arginine being 107 times higher than that of l-arginine. Taking advantage of this property, doxorubicin hydrochloride and the photothermal agent indocyanine green were incorporated into chiral nanoparticles and the high uptake capacity of tumor cells for d-arginine was used to achieve a synergistic anticancer effect of photothermal and chemotherapy. Specifically, the liver-protecting drug silybin can be encapsulated by phosphotyramine-modified β-CD and further assembled with phosphatidylcholine to form nanoparticles [67]. Since liver damage is usually accompanied by elevated alkaline phosphatase, the phosphotyramine group on β-CD can be hydrolyzed, resulting in the release of silybin bound to its cavity, thereby achieving targeted accumulation of the drug. According to a previous report, the hydrolyzate tyramine-modified CD exhibits a high bonding ability to cytotoxic bile acids [68], which can effectively remove excess bile acids and improve exogenous bile acid-mediated cytotoxicity in acute liver injury mouse models. Thus, different drug molecule-modified guests will also promote their applications in *in situ* supramolecular assemblies.

In situ supramolecular assemblies constructed by noncovalent polymerization

Noncovalent supramolecular polymers constructed through host–guest recognition have better reversibility and dynamics and benefit from the hydrophobic cavities of water-soluble macrocycles. These polymers can undergo *in situ* self-assembly under aqueous environments, which establishes the foundation for their therapeutic applications. Macrocyclic compounds with larger cavities are capable of encapsulating two guest molecules simultaneously. When interacting with bifunctional guest compounds, they tend to form one-dimensional nanowires or nanorods [69]. For instance, CB[8] can bond positively-charged anthracene-modified bromophenyl pyridinium salts (ANPY) to form n:n linear polymers with strong NIR fluorescence at 613 nm (Figure 6A) [70]. Due to the tunability of noncovalent interactions, upon photooxidation of the anthracene moiety to anthraquinone under UV irradiation, the assembly underwent an *in situ* transformation from 'head-to-tail' supramolecular polymers to 'head-to-head' 1:2 homologous inclusion complexes, which results in the appearance of strong green phosphorescence in aqueous solution. Notably, this assembly enables precise localization for dual organelle targeting in cellular imaging applications; when cells are stained with ANPY⊂CB[8], initial red fluorescence was observed in the nucleus, and after 2 min of continuous light irradiation, ternary complexes were formed *in situ* that appeared as strong green phosphorescence within the lysosome. In addition to photoregulation, multicolored supramolecular assemblies with controllable topological morphology can also be achieved through the incorporation of water-soluble amphiphilic macrocyclic SC4AD for cascade assembly. The n:n linear polymer, which was formed by anthracene-conjugated phenylpyridine salt encapsulated with CB[8],

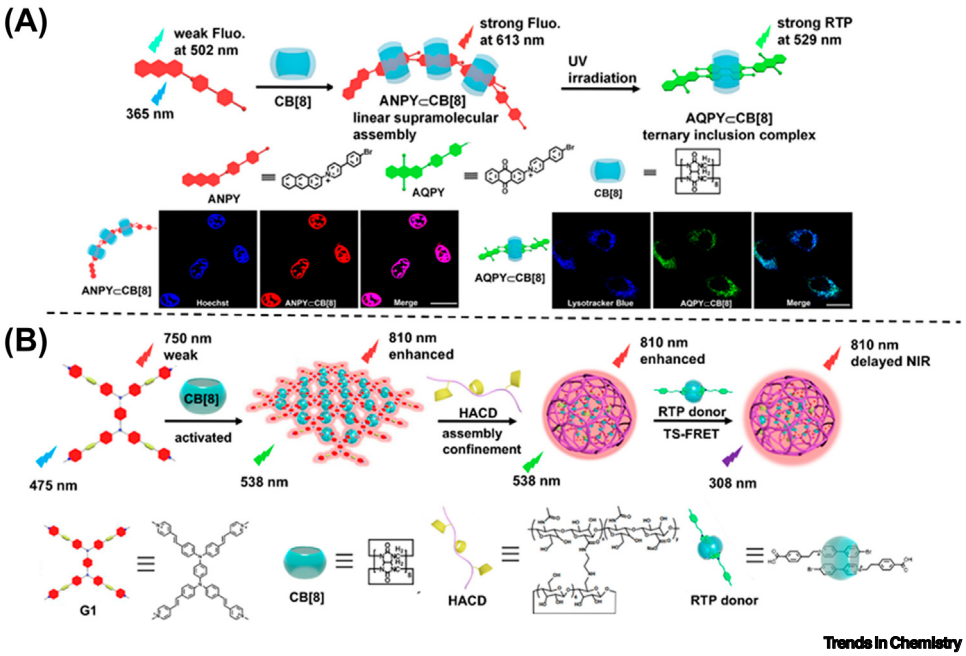

Figure 6. Examples of *in situ* supramolecular assemblies constructed by noncovalent polymerization. (A) Schematic illustration of light-responsive two-cell organelle-targeted supramolecular linear polymers based on CB[8]. (B) Schematic illustration of CB[8]-based supramolecular two-dimensional network-like polymers, which were further co-assembled with hyaluronic acid-modified cyclodextrin (HACD) for cell-targeted imaging with delayed near-infrared (NIR) emission. A, readapted from [70], with permission from American Chemical Society. B, readapted from [29], with permission from American Chemical Society.

transformed to nanorods upon adding the SC4A8, accompanied by an enhancement in red fluorescence at 600 nm [71]. The further restriction of secondary assembly facilitated energy transfer, utilizing Cy5 dye as the energy acceptor to achieve NIR emission at 675 nm with an energy transfer efficiency of up to 71%, which has been successfully applied for targeted imaging of the nuclei in cancer cells. Lou *et al*. designed self-assembled supramolecular nanohelixes utilizing the host–guest interaction between methoxypillar[5]arene and two-armed guests, synergistically driven by metal coordination [72]. The complexation interaction effectively immobilized the distorted conformation of the guest, generating 'twisted sites' that promoted the formation of helical nanostructures and exhibited enhanced yellow–green fluorescence and efficient ROS production due to the restriction of guest molecular motion by dual supramolecular interactions. These properties have been demonstrated to be beneficial for bacterial imaging and photodynamic antibacterial applications.

In contrast to the two-armed molecules, the multi-armed guest molecules are more likely to assemble *in situ* with macrocycles to form reticulated polymers [73,74]. When four-armed phenyl-bridged bis-triphenylamine derivatives were encapsulated by CB[8] in a 1:2 stoichiometric ratio, the ortho-networked two-dimensional nanosheets were formed *in situ*, exhibiting red-shifted fluorescence emission at 810 nm (Figure 6B) [29]. The secondary assembly with the targeting reagent HACD resulted in the formation of three-dimensional nanoparticles, which not only enhanced the NIR fluorescence emission but also facilitated efficient energy transfer when loaded with a phosphorescent donor, producing delayed NIR emission with a Stokes shift of up to 502 nm. This system has been successfully applied for lysosome-targeted cellular imaging. Furthermore, a supramolecular polymer network was reported to be constructed from CB[7], CB[8] co-encapsulated tetraphenylethylene-phenylpyridine derivatives (TPE DPY), where HACD-driven secondary assembly initiated the intramolecular phosphorescence resonance energy

transfer (PRET) process of guest molecules, resulting in NIR delayed fluorescence emission at a wavelength of 700 nm [75]. This single-molecule PRET system has been effectively used for mitochondria-targeted imaging of cancer cells. The encapsulation of CB[8] not only induces single guest molecules to dimerize and form a noncovalent network, but also, the introduction of tripeptides for the ternary co-assembly of heterodimers leads to the chiral transfer [76]. Due to the dynamic reversibility of the macrocyclic supramolecular noncovalent network based on host–guest interactions, the assemblies showed reversible chiral transfer driven by thermal activation. When heated to 65°C, the chiral assembly decomposed and the CD signal at 550 nm gradually disappeared, which gradually recovered after cooling. The supramolecular assemblies exhibited good NIR luminescence properties and can also be used as luminescent materials for cellular imaging to selectively label mitochondria. In addition to bioimaging, we have constructed a protonation-activated self-assembly system *in vivo*, in which tetraphenylpyridine-functionalized (TPE-Py) and CB[8] formed host–guest complexes in lysosomes *in situ* and then self-assembled to form octahedral structures, leading to the instability of lysosomal membranes and release of hydrolytic enzymes to trigger apoptosis [77]. Moreover, the protonated TPE-Py had a high ROS generation efficiency in acidic environments, but almost none in neutral environments, which can damage intracellular biomolecules and trigger oxidative stress. Immunohistochemical staining experiments showed that the number of tumor cell nuclei was obviously reduced, the cancer cells underwent apoptosis, and their proliferation ability was inhibited after the treatment of tumor tissues with the mixture of the supramolecular assemblies.

Supramolecular polymers can also be constructed *in situ* by utilizing the self-assembly of the CD itself with modifications [78]. Yang *et al*. designed supramolecular nanoparticles based on platinum(IV) complex-modified β-CD-ferrocene conjugates (cis-CD-Fc) for synergistic chemotherapy and chemodynamic therapy [79]. Through the one-step supramolecular polymerization process, cis-CD-Fc was used as a supramolecular monomer, which was initiated by polyethylene glycol-modified ferrocene (PEG-Fc) to form linear supramolecular polymers, and then self-assembled into nanoparticles, which were proved to inhibit the growth of tumors and induce apoptosis of tumor cells, and had good biocompatibility and low systemic toxicity. Yu's groups reported camptothecins covalently modified β-CD derivatives via disulfide bonds, which can self-assemble into linear supramolecular polymers and subsequently form nanoparticles under the regulation of iron carboxylate coordination [80]. The supramolecular drugs combined the effects of both chemotherapy and chemodynamic therapy, demonstrating the synergistic effect of cascade potentiation and achieving long-lasting inhibition of tumors in combination with immunotherapy. These findings demonstrated that the *in situ* supramolecular assemblies constructed through noncovalent polymerization can integrate complex functionalities via dynamic molecular recognition. Expanding on these, we foresee that the construction of diverse *in situ* supramolecular assemblies through multivalent interactions between host–guest complexes and ionic liquid or chiral ionic liquid will enable novel biological applications of multifunctional supramolecular systems.

Concluding remarks

From this review article, we can conclude that supramolecular *in situ* self-assembly based on water-soluble macrocyclic compounds depending on noncovalent host–guest interactions in 1:1 or 1:2 ratio, which through the cascade and periodic assemblage, can spontaneously form structurally and functionally unique supramolecular polymers in specific environments, thereby achieving spatial confinement induced and extended luminescence as well as drug encapsulation, and are widely utilized in biological applications. Modifying macrocycles onto polymers can not only provide loading anchors for drugs but also act as nanoreactors. Functionalized guest molecules noncovalently linked to macrocycles through recognition sites can improve their water solubility and biocompatibility, as well as change their aggregation morphology and

Outstanding questions

How can we effectively reduce the leakage of encapsulated drugs outside the target cells?

How can we introduce more functional biomacromolecules like enzyme proteins into *in situ* supramolecular assemblies?

How can we develop supramolecular *in situ* self-assemblies with multi-targeting properties?

How can we accelerate the transformation from laboratory research to clinical application and further promote biological diagnosis and treatment research?

regulate their functions. The supramolecular polymers constructed by *in situ* noncovalent polymerization are not only more dynamic and tunable, but also the two-dimensional network facilitates drug loading and FRET/PRET, which is widely used in bioimaging. Despite significant progress, *in situ* self-assembly of water-soluble macrocycles still faces critical challenges for biomedical applications. Key limitations include the insufficient stability of host–guest complexes under physiological conditions, often leading to premature drug release and degradation. Furthermore, supramolecular drugs frequently encounter immunogenicity issues, while tumor cell drug resistance substantially compromises their therapeutic efficacy. Moreover, the clinical translation of these systems remains limited, with few reported clinical applications to date (see Outstanding questions). With in-depth research, the development of intelligent supramolecular *in situ* assemblies by introducing multiresponsive functional groups is anticipated to enable precise responses to complex physiological environments and, integrated with advanced nanotechnology and biomedical engineering, promises to realize early diagnosis, precise treatment, and real-time monitoring of diseases, thereby bring new breakthroughs in the field of biodiagnosis and treatment [81–84].

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Declaration of interests

The authors declare no conflicts of interest.

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