Cyclodextrin-based bioactive supramolecular assemblies

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Cyclodextrins (CDs) are a class of cyclic oligosaccharides with six to eight D-glucose units linked by α-1,4-glucose bonds, and their capability of forming stable complexes with various organic/inorganic/biological molecules and ions makes them attractive as building blocks for the construction of nano-scale supramolecular systems. This *tutorial review* deals with representative contributions in the construction and the structural characteristics of CD-based supramolecular assemblies as well as their interactions with biologically important substrates. This review is addressed to students and researchers interested in supramolecular chemistry, biochemistry and nanotechnology.

1. Introduction

Within the field of supramolecular chemistry there is an increasing interest focused on the potential applications of cyclodextrin-based systems. Cyclodextrins (CDs), a class of cyclic oligosaccharides with six to eight D-glucose units linked by α-1,4-glucose bonds (Scheme 1), are water-soluble, non-toxic, commercially available compounds with low price, and their structures are rigid and well defined. Most importantly, they possess a hydrophobic cavity that can bind various inorganic/organic/biological molecules and ions in both aqueous solution and the solid state, CDs are extensively studied as not only excellent receptors for molecular recognition but also convenient building blocks to construct nano-structured functional materials, especially bioactive materials. Between the 1970s through the beginning of the 2000s, many successful studies on the bioactivities of natural and simply modified CDs were reported. For example, early researches by Bender,[1] Breslow,[2] Tabushi,[3] and Saenger[4] clearly demonstrated the capability of CDs as enzyme models. From then on, numerous studies showed that, after associating catalytically active groups with CDs, the resultant functionalized CDs can be used as artificial enzyme models to catalyze many biomimetic reactions.[5,6] Moreover, CDs can also increase the utility of enzymes by increasing the availability of insoluble substrates, reducing the substrate inhibition and limiting the product inhibition,[7] can act as drug delivery agents,[8,9] and can sense biological molecules.[10] All these properties are closely related to the inclusion complexation of the hydrophobic cavity of CDs with model substrates. However, the inclusion complexation abilities of natural CDs and simply modified CDs are usually limited, which is inevitably unfavorable to the bioavailability and bioactivity of CDs. To overcome this disadvantage, increasing attention has been paid to the design and construction of CD-based supramolecular assemblies, a class of CD aggregates of nanometer

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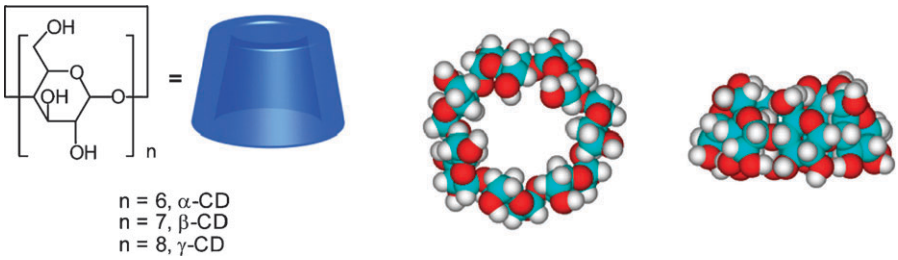

Scheme 1

size. As compared with natural CDs and simply modified CDs, CD-based supramolecular assemblies possess several inherent advantages. First, the CD-based supramolecular assemblies have multiple CD cavities, which can not only strongly bind the substrates through the cooperative binding but also enable the close location between the functional groups of the CD-based supramolecular assemblies and the substrate. Second, the numerous functional groups of CD-based supramolecular assemblies can simultaneously interact with several binding sites of substrates through an integrative effect of a variety of non-covalent interactions. As a joint result of these factors, CD-based supramolecular assemblies can mimic the cooperative "multimode, multipoint" binding often observed in biological systems, and thus exhibit fascinating biological properties. This tutorial review summarizes our recent endeavors and related works by other investigators on CD-based supramolecular assemblies, with a special emphasis on their construction, structural characteristics and their interactions with biologically important substrates.

There are several convenient routes for the construction of CD-based supramolecular assemblies.[11–14] A straightforward way is covalently connecting several CD cavities to a linker molecule or grafting a number of CD cavities on a polymer molecule through nucleophilic displacement, condensation or acylation. Another method is the construction of CD-based supramolecular assemblies through a combined contribution of several non-covalent interactions, and this method is usually used in constructing highly ordered architectures with well-organized topology. Moreover, gold nuclei and carbon nanotubes are also widely applied as templates in the construction of three-dimensional CD-based supramolecular assemblies. The following sections will describe the structural characters of some types of nanometer-sized architectures including CD oligomers, polypseudorotaxanes, polyrotaxanes, nanoparticles, nanocages, hydrogels, *etc*. Owing to the capability of numerous CD cavities and functional groups on associating drug, nucleic acid, protein, and other biological substrates, CD-based supramolecular assemblies are successfully utilized in many biological fields such as fluorescence sensing of biological molecules and ions, drug solubilization and delivery, controlled release, as inhibitors, enzyme mimics, and in DNA cleavage, condensation and transfection, *etc*.

2. Bioactive CD oligomers

CD oligomers are a class of CD-based supramolecular assemblies that comprise several CD cavities linked by functional bridges. Through the cooperative binding of several adjacent CD units, CD oligomers can strongly bind biologically important substrates. Moreover, the functional linker can provide additional interactions with the accommodated biological substrates. The judicious application of these advantages can allow the rational production of bioactive CD oligomers, which will be described below.

2.1 Fluorescence sensing by CD oligomers

Fluorophore-appended CDs show obvious changes of the fluorescence emission upon forming supramolecular assemblies with optically insert molecules or ions. On the other hand, some molecules, which barely fluoresce in solution, also present appreciable emission upon forming a supramolecular assembly with functional CD derivatives. These unique fluorescence behaviors consequently enable CDs to act as efficient fluorescence sensors for biologically important substrates. Upon forming stable supramolecular assemblies with steroids, β-CD dimers with biquinolino linkers and oxamido bisbenzoic carboxyl linkers as well as their metal complexes show remarkable fluorescence enhancements and thus can be used as efficient fluorescence sensors for steroids.[15,16] The increased fluorescence is attributed to the changes of location and orientation of biquinolino or oxamido bisbenzoic carboxyl moieties in these dimers upon forming supramolecular assemblies, which consequently leads to the increased microenviromental hydrophobicity and/or steric shielding around the fluorophore (Fig. 1). As an example, a γ-CD derivative bearing a 4-amino-7-nitrobenz-2-oxa-1,3-diazole moiety can selectively sense α-amylase, giving decreased fluorescence in the presence of *Aspergillus oryzae* α-amylase due to the amylase-induced hydrolysis of the γ-CD, but showing little fluorescence changes with organic compounds. A calibration curve of fluorescence intensity changes *versus* the α-amylase concentration suggests that the sensitivity of this system (>1 μg mL^{-1}) is suitable for clinical samples, since the concentration of α-amylase in human serum is 0.5–1.6 mg mL^{-1} and γ-CD can be hydrolyzed by human salivary and pancreatic α-amylases at an appreciable rate.[17]

The fluorescence intensities of some biological substrates are sensitive to the changes of their microenvironment. That is, they barely fluoresce in a hydrophilic microenvironment but emit strong fluorescence in a highly hydrophobic one. Therefore, when these substrates are located in a highly hydrophobic microenvironment, such as the hydrophobic CD cavities, to form supramolecular assemblies, significant fluorescence emission can be observed. For example, although L-tryptophan, adenine, guanine and xanthine barely fluoresce in solution, the supramolecular assemblies formed by

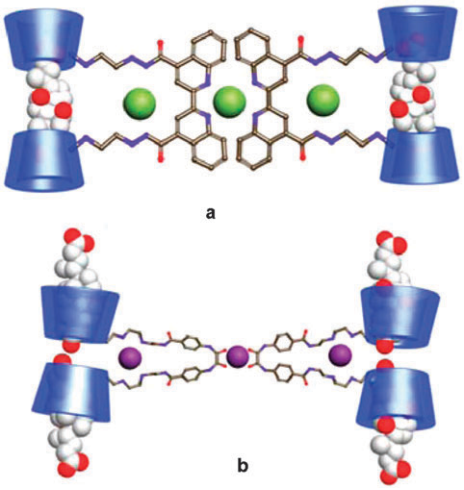

Fig. 1 Schematic representation of supramolecular assemblies formed with steroids by metallobridged β-CD dimers possessing biquinolino linkers (a) or oxamido bisbenzoic carboxyl linkers (b).

N,*N*′-bis(2-(2-aminoethylamino)ethyl)malonamide-bridged bis(β-CD) with these substrates show significant fluorescence, and the emission intensities are strong enough to be readily distinguished by the naked eye even at a low concentration (Fig. 2).[18] Besides molecules, some biologically important ions can be also efficiently detected *in vivo* by forming fluorescent supramolecular assemblies with CD derivatives. Due to the $3d^{10}4s^0$ electronic configuration, Zn^{2+} provides no appreciable spectroscopic or magnetic signals and thus is difficult to detect in biological systems by most of the common analytical techniques. However, through the very strong coordination of Zn^{2+} with aminobenzenesulfonamidoquinolino-β-CD ($\log K_s = 12.4$), the resultant supramolecular assembly emits an intense greenish fluorescence at 518 nm, and its intensity reaches 5.7 times as high as that of free aminobenzenesulfonamidoquinolino-β-CD. Significantly, this Zn^{2+}-promoted fluorescence response is available over a wide pH range and not obviously affected by other biologically important cations such as Na^+, K^+, Mg^{2+} and Ca^{2+} under physiological conditions. Further investigations by fluorescence microscopy using yeast as model cells show that originally non-luminescent yeast cells present a very weak background fluorescence without Zn^{2+}, but exhibit a strong green fluorescence that can be easily detected by a fluorescence microscope in the presence of Zn^{2+} (Fig. 3). These results may provide a new method to detect Zn^{2+} released from stimulated cells.[19]

The application of fluorescence sensing of CD-based supramolecular assemblies can also extended to the field of DNA migration. Besides acting as an inhibitor for DNA topoisomerase and DNA cleavage enzymes owing to its capability of inducing the aggregation of DNA, a β-CD-based $Ru(phen)_3$ complex can also efficiently detect the translocation of DNA into 293 T cells (human embryonic kidney cell line) owing to its good luminescent property. The fluorescence microscopic images of normally non-luminescent 293 T cells in the presence of supramolecular assembly/DNA mixtures by comparison with the corresponding phase contrast images show that nearly all of cells are luminescent under the fluorescence microscope,

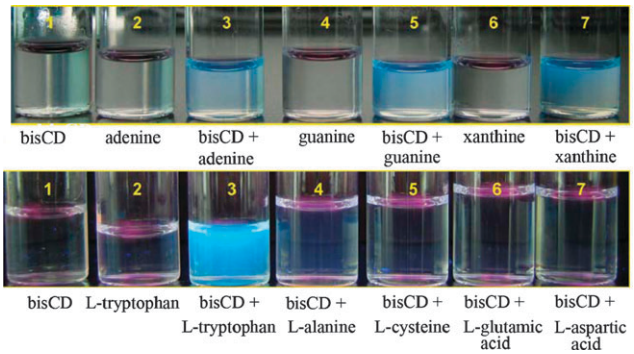

Fig. 2 Visible emission observed from samples of *N*,*N*′-bis-(2-(2-aminoethylamino)ethyl) malonamide-bridged bis(β-CD) and various biochemical molecules.

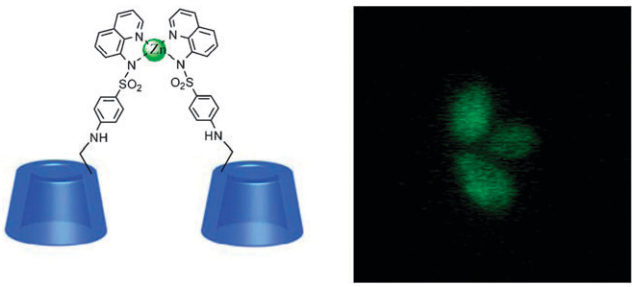

Fig. 3 Fluorescence microscopic images of aminobenzenesulfonamidoquinolino-β-CD-stained yeast cells with the addition of Zn^{2+}.

indicating a high DNA translocation efficiency of the supramolecular assembly (Fig. 4). It should be noted that, in the supramolecular assembly-mediated DNA translocation process, the β-CD cavity of supramolecular assembly, which has a good capability to accommodate hydrophobic molecules, is unoccupied. Therefore, the supramolecular assembly may also have the potential to carry active drug molecules into cells, many of which are usually hydrophobic, and only enter cells with difficulty.[20]

2.2 Solubilization and delivery of drug molecules by CD oligomers

Possessing CD cavities that are water–soluble, nontoxic and able to bind various drug molecules, CD-based supramolecular assemblies have numerous potential applications in medicinal chemistry, for example, as drug solubilizers and carriers. The water solubility of paclitaxel, an important antitumor drug, is only 0.7–30 μg mL^{-1}, but this is raised to a level of 2 mg mL^{-1} (calculated as paclitaxel residue) in a supramolecular assembly formed by a tetraethylenepentaamino-bridged bis(β-CD) and two paclitaxel complexes. Biological activity tests using the MTT cytotoxicity assay show that the supramolecular assembly at a concentration of 1×10^{-6} mg mL^{-1} exhibits an inhibitive ability of 57.7% for K562 erythroleukemia after 72 h, which is even higher than the corresponding value of the parent paclitaxel under the same conditions.[21] Another successful example of CD-based supramolecular assemblies in tumor therapy is the supramolecular assembly formed between a bis(β-CD) with a photocleavable linker and a phthalocyanine-based photosensitizer that generates singlet

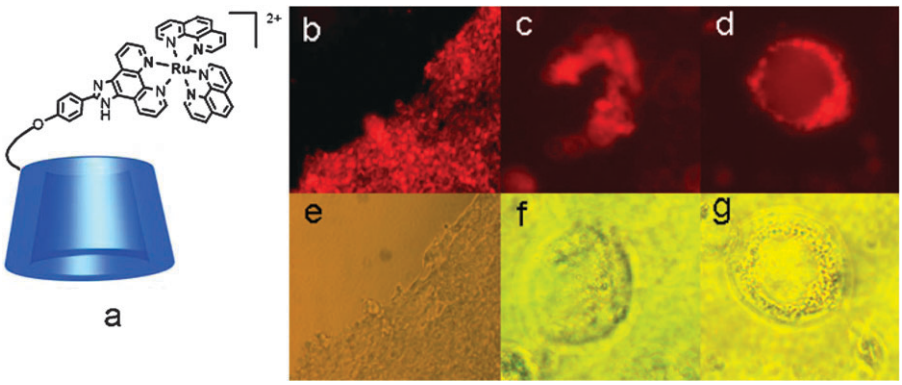

Fig. 4 Fluorescence microscopic images of natural cultured 293T cells in the presence of β-CD-Ru(phen)$_3$/DNA mixtures; (c, d) enlarged images of (b), (e–g) corresponding phase contrast images of (b–d).

oxygen on irradiation. When this supramolecular assembly is irradiated in the tumor region, the generated singlet oxygen cleaves the linker of bis(β-CD). As a result, the β-CDs are released, and the photosensitizer is delivered to the affected cells. Interestingly, the destruction of the supramolecular assembly causes more of it to diffuse into the light path, which consequently concentrates the photosensitizer in the tumor region.[22] Moreover, with an affinity in the low nanomolar region, the supramolecular assembly formed between naphthalenedithiolyl-bridge bis(β-CD) and ^{64}Cu–1,7-(4-*tert*-butylphenylmethyl)cyclen can also be used as a receptor/ligand system for tumor pretargeting, wherein the bis(β-CD) is concentrated at the tumor site by the conjugation *via* a covalent bond to a monoclonal antibody, to mimic the often utilized system of avidin or streptavidin/biotin.[23]

2.3 Noncatalytic biomimesis by CD oligomers

Other than the enzymatic catalysis properties that are presented by many simply modified CDs,[6] CD-based supramolecular assemblies exhibit some different biological functions, such as mimicking of noncatalytic enzymes. Supramolecular assemblies based on organoselenium-modified β-CDs containing 1,2-benzisoselenazol-3(2*H*)-one moieties show satisfactory superoxide dismutase (SOD) activities of up to 121–330 U mg^{-1} and the glutathione peroxidase (GPX) activities of up to 0.34–0.86 U μmol^{-1}.[24] Moreover, a supramolecular assembly formed by 2,2′-ditellurobis(2-deoxy-β-CD) and 4-nitrobenzenethiol (NBSH) is highly efficient in the reduction of ROOH in the NBSH assay system, and its second-order rate constant for NBSH is similar to that of native glutathione peroxidase (GPX).[25] Moreover, the supramolecular assembly constructed from a Ru(II)-porphyrin-bridged bis(β-CD) can efficiently mimic the carotene dioxygenase enzymes that cleave β,β-carotene to provide retinal as a precursor for retinol (vitamin A). In this case, the end groups of β,β-carotene are bound by β-CD cavities, and a lateral movement of β,β-carotene within the binding pocket exposes only three double bonds of β,β-carotene to the reactive Ru=O group of the bis(β-CD), leading to a 41% cleavage selectivity of the central double bond to form retinal. However, by changing β,β-carotene to carotenoid where one of the cyclohexene end-groups of β,β-carotene is replaced by an *ortho*-dimethylphenyl group, the cleavage selectivity of the central double bond is almost exclusive. This demonstrates the important relationship between the binding ability of substrates and the cleavage selectivity.[26] It should be noted that, in all of these systems, the effective binding of substrates by β-CD cavities and the cooperation between the catalytic groups and the substrate-binding sites (β-CD cavities) play important roles in the enzyme mimic.

2.4 Interactions of CD oligomers with peptides and proteins

For CD oligomers possessing oligoethylenediamino fragments in the linkers, an inherent advantage is their efficient binding towards peptides. In neutral media, –NH– units in the oligo(ethylenediamino) fragments are partly protonated. Therefore, the electrostatic interactions between the protonated amino groups (–NH$_2^+$–) in the linker and the anionic carboxylate group of peptides, as well as hydrogen-bond interactions between the carbonyl, carboxyl and amino groups in peptides and oligoethylenediamino fragments in the linker, jointly strengthen the association of peptides with CD oligomers. For CD oligomers with metal centers, the coordinated metal centers also provide additional binding interactions towards peptides through heteroatom–metal chelation effects and/or electrostatic interactions. As a cumulative result of these factors, CD oligomers show moderate to strong binding abilities towards oligopeptides with a K_s range of 10^2–10^4 M^{-1}.[11] In addition, CD oligomers can also block protein–protein interactions. A comparative study on the inhibitor activities of 11 β-CD dimers against 8 enzymes shows that CD dimers in which two β-CDs are linked on the secondary face by a pyridine-2,6-dicarboxylic group can inhibit the activity of L-lactate dehydrogenase and citrate synthase at least in part by the disruption of protein–protein aggregation.[27]

3. Bioactive one-dimensional supramolecular assemblies

3.1 CD-based polypseudorotaxanes and polyrotaxanes

CD-based polypseudorotaxanes are a type of supramolecular assembly that consists of a long-chain molecule (axle component) and several CD molecules (wheel component).[12] One of the generally used methods to obtain CD-based polypseudorotaxanes

was established by Harada.[28] In this method, CD-based polypseudorotaxanes are constructed through the threading of polymers or polyelectrolytes with CDs and stabilized by the hydrogen bonding between the adjacent CD cavities as well as the non-covalent interactions between the long-chain molecule and the threaded CD cavities. After introducing bulky terminals, such as bulky organic or organometallic groups, at the chain ends to prevent the dethreading of CDs, CD-based polypseudorotaxanes can be converted to CD-based polyrotaxanes. When the CD derivatives with bioactive substituents are used as the wheel components, bioactive CD-based polypseudorotaxanes or polyrotaxanes can be obtained. A first example of these bioactive CD-based supramolecular assemblies is the DNA reactivity of polypseudorotaxanes constructed by the threading of the CD units with polycations and/or fused-ring aromatic substituents onto the polymer chain. As a good bioactive precursor, anthryl-modifed CD can be used as chemically switched DNA intercalators, giving decreased fluorescence with the addition of DNA in the presence of 1-adamantanol.[29] After threading the anthryl-modified β-CDs onto the poly(propylene glycol) bis(2-aminopropylether) (PPG–NH_2, MW $\approx$2000) chains, the obtained CD-based polypseudorotaxanes with 9–10 anthryl grafts not only increase the helix melting temperature, *i.e.* the temperature at which the double helix denatures to single stranded DNA, by 6 °C, but also condense the originally loose free DNA to solid particles with an average diameter of *ca.* 100 nm. The molecular modeling studies show that, differently from the case of anthryl-modified β-CD where the anthryl group intercalates in either the minor or the major DNA groove, the anthryl groups in polypseudorotaxanes intercalate in both the minor and major DNA grooves. Therefore, the driving force of the DNA condensation should be not only the electrostatic interactions between the protonated amino groups in polypseudorotaxanes and the negatively charged phosphates in DNA, but also the intercalation of multiple anthryl groups into the DNA grooves (Fig. 5).[30]

Another important example of these bioactive CD-based supramolecular assemblies is cationic CD-containing polymers or polyrotaxanes constructed by threading cationic CD derivatives onto polymer backbones, showing good DNA binding ability, low cytotoxicity, and high gene transfection efficacy, especially by cationic CD-based polyrotaxanes.[31] For example, possessing a high cationic density, a type of polyrotaxane constructed from oligoethylenimine-grafted β-CDs threading onto the polymer chain, shows the high gene transfection efficiency with and without serum (Fig. 6), comparable to that of branched PEI (25 K), one of the most effective gene-delivery polymers studied to date. Moreover, the transfection efficiency of these cationic polyrotaxanes in most cases increases with the elongation of oligoethylenimine grafts on the β-CDs.[32]

Besides interacting with DNA, CD-based polyrotaxanes also contribute to the acceleration of physicochemical interactions with the plasma membrane and the intracellular metabolism. Polyrotaxanes constructed by threading many hydroxypropylated-α-CDs onto a poly(ethyleneglycol) (PEG) chain and ending with a L-phenylalanine moiety *via* a peptide linkage can inhibit the cytoplasmic calcium increase in platelets, increase the plasma membrane fluidity of red blood cell (RBC) ghosts, and elevate the cytoplasmic cyclic AMP levels in platelets by *ca.* 20%. Moreover, these polyrotaxanes degrade into non-interactive components (hydroxypropylated-α-CD, PEG and L-Phe) within an acceptable period of time and will be naturally removed from the body, which consequently enable its application in the fabrication the blood-contacting devices.[33]

Different from the CD oligomers that work as drug carriers through the association/release of drug molecules by CD cavities, biodegradable CD-based polyrotaxanes are another type of drug and gene delivery systems related to CD-based supramolecular assemblies.[31] In these polyrotaxanes, the drug-conjugated CDs or cationic CDs are threaded on a polymer chain and then capped by stoppers *via* biodegradable linkages. When the linkages are degraded under special conditions, the polyrotaxane is destroyed, and the drug–CD conjugate or DNA is released. For example, the polyrotaxane constructed by threading dimethylaminoethyl-modified α-CDs

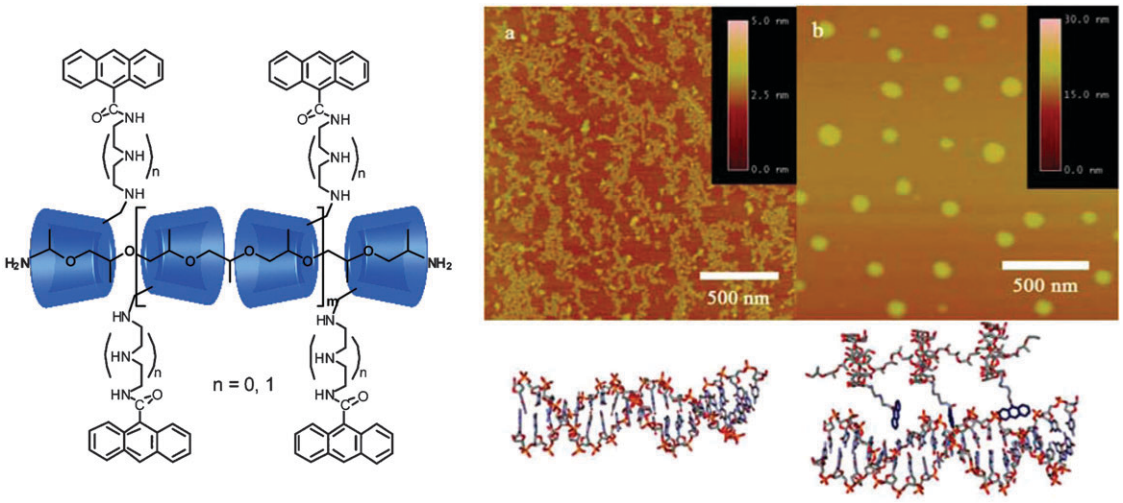

Fig. 5 Molecular structure of CD-based polypseudorotaxanes with anthryl grafts and AFM images of (a) free calf-thymus DNA and (b) condensed DNA induced by CD-based polypseudorotaxane with anthryl grafts.

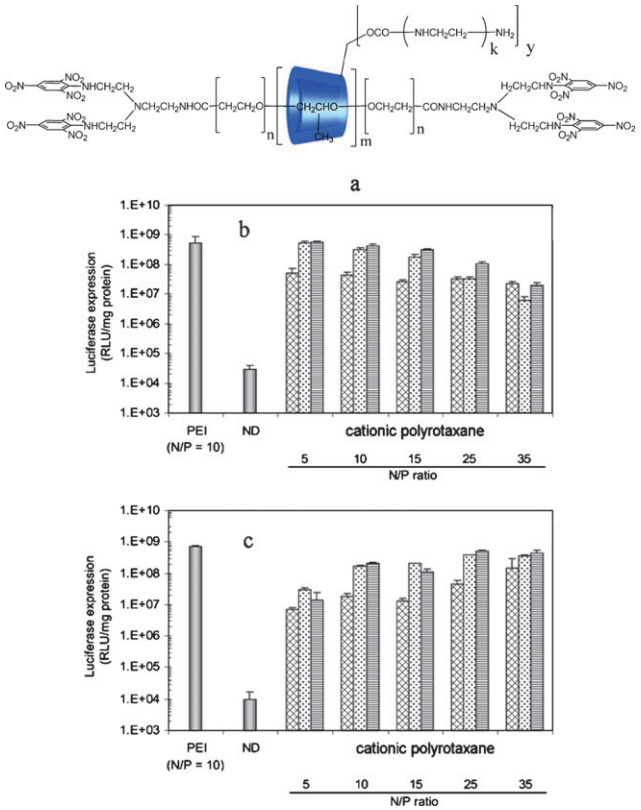

Fig. 6 Schematic representation of cationic polyrotaxane (a) and *in vitro* gene transfection efficiency of cationic polyrotaxane/DNA in HEK293 cells in comparison with that of PEI (25 K) or naked DNA (ND) in (b) the absence and (c) the presence of serum. The transfection efficiency of PEI (25 K) was obtained with the optimal N/P ratio of 10.

onto a PEG (M_n = 4000) chain and ending with benzyloxycarbonyl tyrosine *via* disulfide linkages can condense pDNA to a tightly packed particle with a diameter of *ca.* 178–189 nm. However, under a reducible condition in the presence of 10 mM dithiothreitol (DTT) and dextran sulfate as a counter polyanion, the disulfide linkages are cleaved and the originally condensed pDNA is released through the inter-exchange with dextran sulfate polyanions.[34] Because different parts of the human body have different pH and different enzymes, the pH adjustment and the catalysis by special enzymes are also utilized in the controlled release of drugs from the degradable polyrotaxanes, showing either association or dissociation towards drugs under different conditions.

In most cases, the CD units in a polypseudorotaxane can spin around and move back and forth along the polymer backbone. Benefiting from these movements, the CD units of a polypseudorotaxane can span certain distances and dynamically present their functional groups to adjust to the relative stereochemistries of binding sites of biological substrates. For example, the polypseudorotaxane constructed from lactoside-CDs threaded onto a linear polyviologen chain exhibits an ability to inhibit galectin-1-mediated T-cell agglutination up to 10–30 times higher than that of native lactose (Fig. 7).[35,36] In these supramolecular assemblies, the flexible and dynamic presentation of binding sites on CDs resulting from the CD rotation around and the limited translation along the polymer

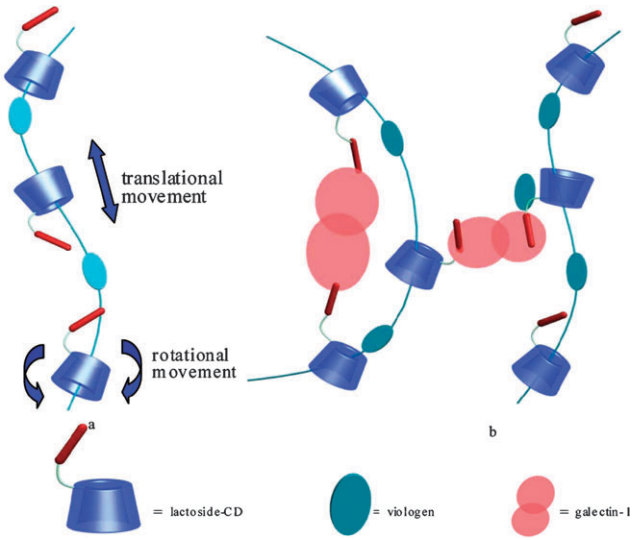

Fig. 7 (a) Movement modes of CDs in a polypseudorotaxane and (b) possible binding modes of pseudopolyrotaxane with galectin-1.

chain play the important role in the protein–carbohydrate interactions.

3.2 CD-based nanowires

Different from polypseudorotaxanes and polyrotaxanes that are composed of a long-chain component and many CD units, CD-based nanowires are another type of one-dimensional supramolecular assembly that consists of many CD derivatives combined by equivalent linker components.[11] In a typical example, a nanowire constructed through the end-to-end intermolecular inclusion complexation of Pt(II)-coordinated 6,6′-*O*-phenylenediseleno-bridged bis(β-CD)s with C_{60} molecules exhibits a regular linear array according to scanning tunneling microscopy (STM) and transmission electron microscopy (TEM) images. Biological activity tests under visible light irradiation or in the dark indicate that the nanowires can cleave the closed supercoiled form of DNA into the nicked circular form upon incubation under visible-light irradiation but showed no cleavage ability in the dark (Fig. 8).[37] Herein, a singlet oxygen mechanism is responsible for the DNA-cleavage reaction. The C_{60} moieties in the nanowire are located close to the guanosine positions of DNA and under visible-light irradiation singlet oxygen ($^1O_2$) is sensitized by the photoexcitation of C_{60}. Then, the sensitized singlet oxygen reacts with the guanosines in DNA by either [4 + 2] or [2 + 2] cycloaddition to the five-membered imidazole ring of the purine base, thus cleaving the DNA. This cleavage mechanism can be verified by the detection of the singlet oxygen by EPR spin-trapping using a $^1O_2$-trapping agent.[18]

4. Bioactive two- and three-dimensional supramolecular assemblies

4.1 Two-dimensional polypseudorotaxanes

As a successful improvement of polypseudorotaxane polycations, a two-dimensional cationic polypseudorotaxane is

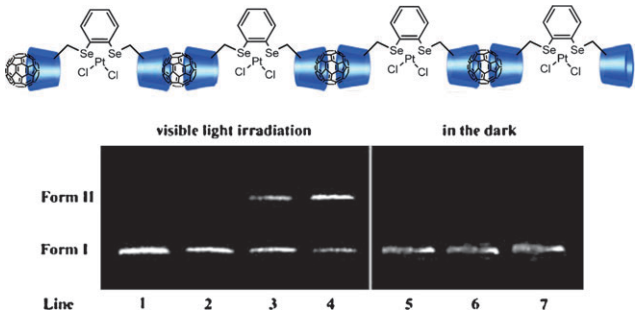

Fig. 8 Schematic representation of nanowire and agarose gel electrophoretic patterns of DNA and nicked DNA. Line 1: no reagent in Tris-HCl buffer (pH 7.4); lines 2 and 5: with bis(β-CD); lines 3 and 6: with Pt(II)-coordinated bis(β-CD); lines 4 and 7: with nanowires.

constructed by threading 6-[(6-aminohexyl)amino]-6-deoxy-β-CD dichlorides on the polymer backbone, followed by complexing cucurbit[6]urils on the arms of modified CDs. Interestingly, this two-dimensional cationic polypseudorotaxane displayed controllable DNA condensation ability by adjusting the amount of cucurbit[6]urils in the polypseudorotaxanes. The DNA condensation ability of this supramolecular assembly reaches its highest efficiency when containing 70% of cucurbit[6]urils (Fig. 9).[38] Further investigations by agarose gel electrophoresis, ethidium bromide (EB) displacement, and atomic force microscopic (AFM) experiments demonstrate that the effective charges of polypseudorotaxane interacting with DNA and the rigidity of polypseudorotaxane changing with the addition of CB[6]s, jointly lead to the unusual DNA condensation ability of the two-dimensional polypseudorotaxane.

4.2 CD-based supramolecular hydrogels

The threading of CDs, particularly by α-CDs, onto parts of long polymers or co-polymers (PEO, PEO-PPO-PEO, PEO-PHB-PEO, *etc.*) of high molecular weight can result in the formation of supramolecular hydrogels, another type of drug delivery system related to CD-based supramolecular assemblies,[31] and various concentrations of CD can be used to formulate different hydrogels. Generally, the viscosity of the supramolecular hydrogel greatly diminishes when it is agitated, but the diminished viscosity eventually restores towards its original value within a period of time when there is no further agitation. This property, along with the thixotropic property, jointly enables the potential applications of supramolecular hydrogels as an injectable drug delivery system. That is, after incorporating the drug into the hydrogel in a syringe at room temperature without any contact with organic solvents, the drug-loaded hydrogel can be injected into the tissue under pressure because of the thixotropic property, and then serves as a depot for the controlled release of drug.

4.3 CD-based supramolecular assemblies mediated by gold

Using some inorganic or organic nanostructures as templates, CD-based three-dimensional supramolecular assemblies can be conveniently constructed through covalent or non-covalent association of modified CD systems with these templates. Through the strong Au–S binding, thio- or polythio-modified CDs can be absorbed on the surface of gold to form three-dimensional supramolecular assemblies. In a typical example, possessing many hydrophobic cavities at the outer space, a supramolecular assembly constructed by adsorbing oligo-(ethylenediamino)-CDs on gold nanoparticles shows not only good ability as a vector for DNA binding but also moderate plasmid transfection efficiency as a carrier into cultivated cells *in vitro*, which are sufficiently investigated by means of circular dichroism spectroscopy, transmission electron microscopy, and visual GFP expression (Fig. 10).[39]

In addition, the amino-terminated polypseudorotaxane can also attach to the surface of different gold particles to form three-dimensional nanocages through the electrostatic interactions between the amino terminals of polypseudorotaxane and the gold nuclei, and the number of polypseudorotaxane

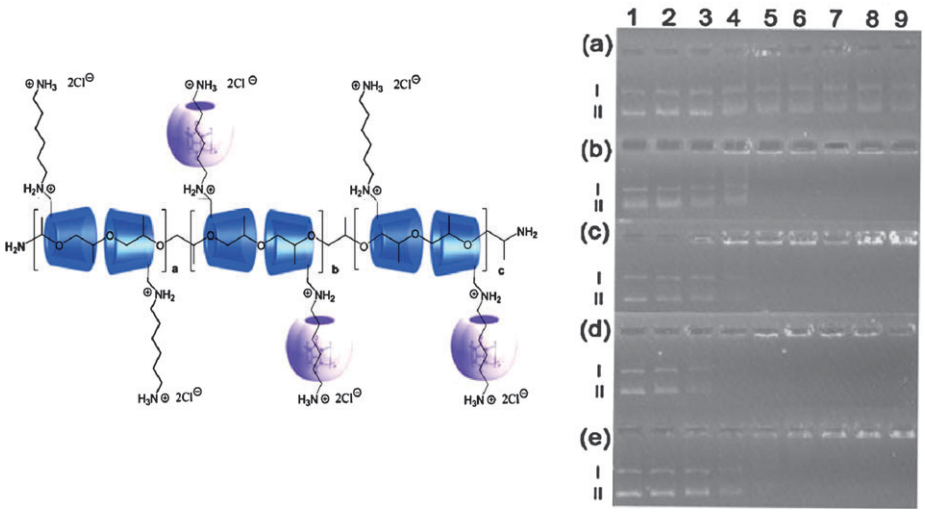

Fig. 9 Schematic representation of two-dimensional polypseudorotaxane and agarose gel electrophoresis patterns of the DNA condensation induced by two-dimensional polypseudorotaxane bearing (a) 0, (b) 20, (c) 40, (d) 70 and (e) 100% of CB[6]s. Lane 1, DNA alone; lanes 2–9, DNA + two-dimensional polypseudorotaxane. The molar ratios between two-dimensional polypseudorotaxane and DNA nucleotide increase from lane 1 to 9.

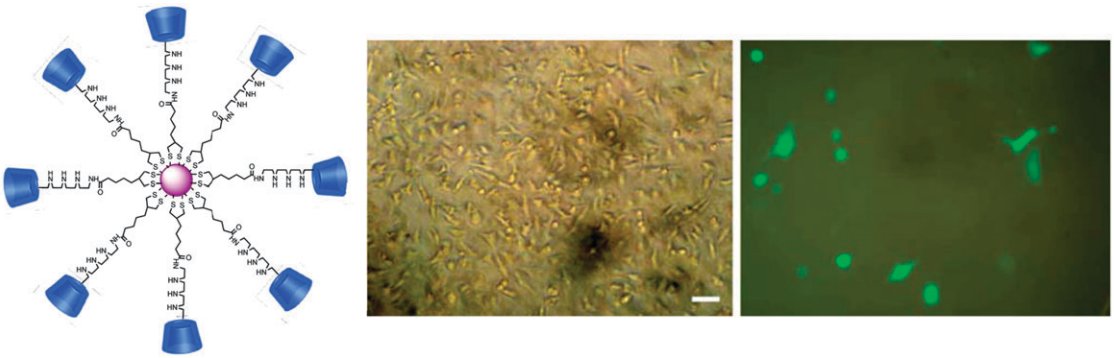

Fig. 10 *In vitro* DNA transfection of MCF-7 cells by oligo(ethylenediamino)-CD-modified gold nanoparticles. Scale bar: 20 μm.

on each of nanoparticles tends to decrease, while the average surface area occupied by one polypseudorotaxane and the average nanocage size increase, with the elongation of the polypseudorotaxane used. Interestingly, this type of nanocage constructed by the attachment of numerous L-Try-CD-based polypseudorotaxanes onto the surface of gold nanoparticle only gives a weak DNA cleavage ability. However, after being saturated by C_{60}, the nanocage exhibits much higher DNA cleavage activity under visible light irradiation, and most of the closed supercoiled DNAs are cleaved to the nicked circular DNA (Fig. 11).[40]

Other than the C_{60}-containing supramolecular assemblies on gold, another type of supramolecular assembly constructed by covalently grafting of gold nanoparticles to thioCD-based polypseudorotaxanes also shows a good photoinduced DNA cleavage ability, which is also attributed to a singlet oxygen mechanism. That is, when the absorption of red light induces a gold d–d transition, the excitation at shorter visible wavelengths leads to the sulfur-to-gold charge-transfer band excitation at the initial step of photocleavage. The excitation energy is subsequently transferred to the ground state oxygen molecules to produce singlet oxygen that cleaves the DNA.[41]

The strong binding of substrates by CD derivatives also enables the thus formed supramolecular assembly on gold to mimic antigen–antibody recognition. A supramolecular nanostructure formed by the multi-ordered association of a divalent bis(adamantyl)-biotin linker, streptavidin, and biotinylated protein on β-CD monolayers is shown in Fig. 12. In this system, the divalent linker is bound to the β-CD monolayer on gold, followed by the subsequent attachment of streptavidins. Then, the antibodies are specifically bound to the streptavidin layer *via* a biotinylated protein or *via* a biotinylated antibody. Significantly, the immobilization of the antibodies to the β-CD monolayers can be used to create the platforms for lymphocyte cell count purposes, wherein an optimized orientation of the antibodies leads to the highest binding specificity of the immobilized cells.[42]

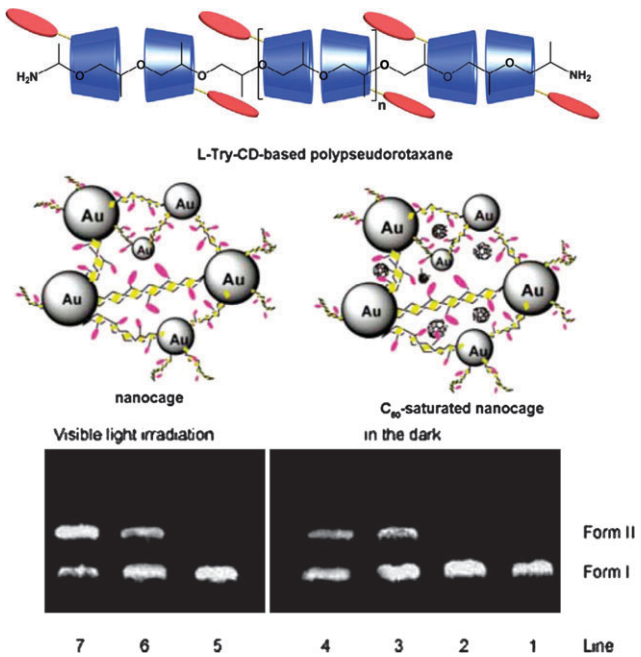

Fig. 11 Schematic representation of L-Try-CD-based polypseudorotaxane nanocage, C_{60}-saturated nanocage and agarose gel electrophoretic patterns of plasmid DNA. Line 1: no reagent in Tris-HCl buffer (pH 7.4); lines 2 and 5: with L-Try-CD-based polypseudorotaxane; lines 3 and 6: with nanocage; lines 4 and 7: with C_{60}-saturated nanocage.

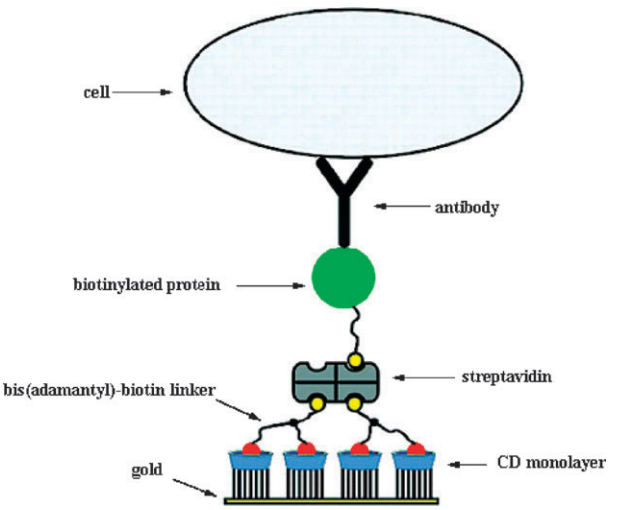

Fig. 12 Multi-ordered structure of CD-based supramolecular assembly for antibody recognition and cell counting.

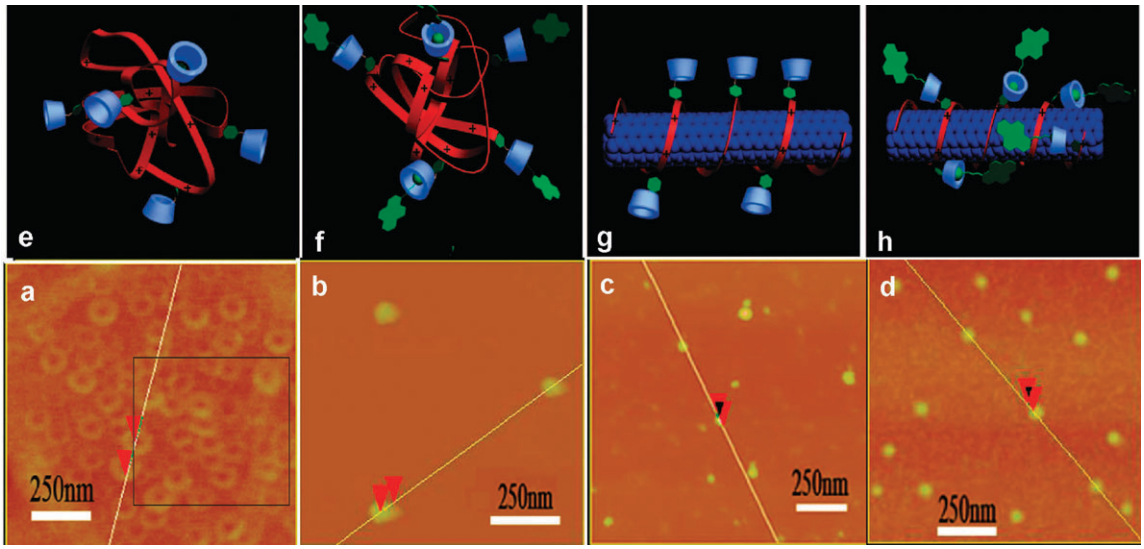

Fig. 13 AFM images (a–d) of DNA condensation induced by CD-modified chitosan (e), chitosan/pyrene (f), nanotube/chitosan (g) and nanotube/chitosan/pyrene (h).

4.4 CD-based supramolecular assembly mediated by carbon tubes

In addition to gold particles, carbon nanotubes are also used as templates to construct three-dimensional CD-based supramolecular assemblies. For example, many linear macromolecules, including organic polymers and biomacromolecules, are able to non-covalently couple with carbon nanotubes through wrapping or adsorption. Therefore, by introducing CD units to these macromolecules followed by the wrapping or the adsorbing process, the nanotube/CD polyad supramolecular assemblies can be constructed conveniently. In a typical example, a β-CD-modified chitosan shows a moderate DNA condensation ability and is able to condense free DNA chains to uniform hollow loops. After associating the adamantanyl pyrene molecules to the β-CD-modified chitosan, the resultant dyad with numerous exposed pyrene grafts is much more effective in condensing DNA than the modified chitosan, and the free DNA chains are condensed to infarcate particles with an average diameter of *ca.* 200 nm by the dyad rather than the hollow loops by the modified chitosan. The enhancement of DNA condensing efficiency is ascribed to the cooperative contribution of aromatic pyrenes and inherent $-NH_3^+$ cations on the chitosan. Interestingly, by wrapping the modified chitosan to the carbon nanotube, the resultant dyad can condense the free DNA to compact particles with an average diameter of *ca.* 80 nm. The wrapping of modified chitosan on the carbon nanotube rearranges the modified chitosan on the surface of the carbon nanotube as highly dispersed polymers, which enables more active $-NH_3^+$ cations interacting with DNA grooves. Inspired by the improved effects of the DNA condensation shown by chitosan/pyrene dyads and nanotube/chitosan dyads, the nanotube/chitosan/pyrene triad was tested as a combinatorial vector and showed a promoted DNA condensation ability as compared with that of nanotube/chitosan dyad (the DNA particles were enlarged from 80 nm wide in the nanotube/chitosan dyad to 160 nm wide in the nanotube/chitosan/pyrene triad) though results were not superior to that of the chitosan/pyrene dyad (Fig. 13).[43]

Because the surface of nanotube is hydrophobic, it can be readily wrapped by single-stranded DNA through hydrophobic and π–π stacking interactions, while it hardly interacts with the double-stranded DNA in which the hydrophilic sites (phosphates) are exposed on the surface. However, after wrapping a anthrylCD-based polypseudorotaxane on the surface of carbon nanotube, the resultant nanotube/polypseudorotaxane supramolecular assembly shows a good ability of wrapping and cleaving double-stranded DNA (Fig. 14).[44] Thermodynamically, the association of the nanotube/polypseudorotaxane supramolecular assembly with DNA gives a favorable enthalpic contribution and a large entropic loss ($\Delta H^\circ = -29.6$ kJ mol^{-1}, $T\Delta S^\circ = -5.4$ kJ mol^{-1}), and the adsorption of CDs onto the carbon nanotube as well as the intercalation of anthryl groups into the DNA grooves may play an important role in the DNA wrapping. Interestingly, a close comparison of AFM images of nanotube/polypseudorotaxane supramolecular assemblies with and without DNA show that several DNA duplexes may wrap onto the nanotube/polypseudorotaxane supramolecular assembly rather than only one.

5. Conclusion and outlook

It is clear that CD-based bioactive supramolecular assemblies can be constructed through the covalent syntheses of bioactive CD species as building blocks *via* the disruption and the construction of covalent bonds, followed by the molecular assembly of building blocks *via* a simultaneous contribution of several noncovalent interactions. Upon interacting with biological substrates, a CD-based supramolecular assembly provides many CD cavities as hydrophobic binding sites and numerous functional groups as versatile binding sites, and these binding sites can simultaneously associate with the corresponding sites of substrates, which is similar to the cooperative "multimode, multipoint" bindings often observed in biological systems. As a result, CD-based supramolecular assemblies thus obtained always exhibit significant selective

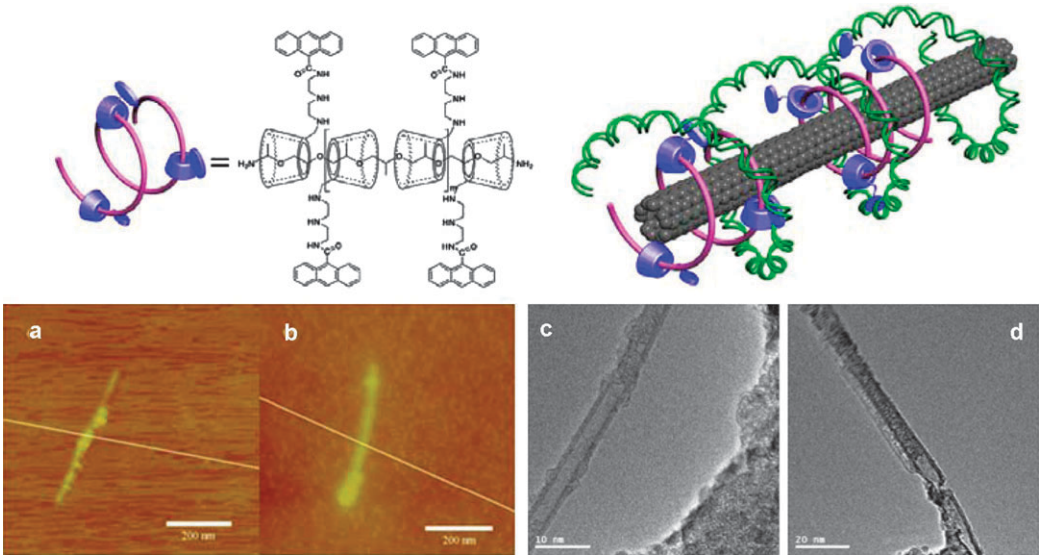

Fig. 14 Schematic representation of DNA wrapping by a nanotube/polypseudorotaxane supramolecular assembly, AFM images of a nanotube/polypseudorotaxane supramolecular assembly in the absence (a) and the presence (b) of DNA, and TEM images of a nanotube/polypseudorotaxane supramolecular assembly in the absence (c) and the presence (d) of DNA.

binding abilities towards biological substrates through simultaneous contributions of these factors, and this fascinating property enables many successful biological applications of CD-based supramolecular assemblies.

In this review it has been shown that there are many examples of CD-based supramolecular systems in which the process of molecular assembly can be fully controlled, resulting in bioactive materials with fascinating functions. In this regard, the guiding principle for designing CD-based bioactive supramolecular assemblies can be approved in the light of accumulated research accomplishments summarized here, and the next target of studies on bioactive supramolecular assemblies may be to establish well-organized supramolecular systems through the judicious alignments of bioactive building blocks in an ordered manner. Such highly ordered systems may be also designed through a combination of CD-based binding blocks with the fragments of biological substrates, and the resulting systems may be expected to exhibit enhanced bioactivity as a consequence. The past two decades have witnessed a significant harvest in CD-based bioactive supramolecular assemblies. However, we believe that the exciting findings and potential of CD-based supramolecular assemblies are only beginning to be discovered.

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