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ABSTRACT

Macro biomolecules are of vital importance in regulating the biofunctions in organisms, in which proteins (including peptides when mentioned below) and nucleic acids (NAs) are the most important. Therefore, these proteins and NAs can be applied as “drugs” to regulate the biofunctions from abnormal to normal. Either for proteins and NAs, the most challenging thing is to avoid the biodegradation or physicochemical degradation before they reach the targeted location, and then functions as complete functional structures. Hence, appropriate delivery systems are very important which can protect them from these degradations. Cyclodextrins (CDs) based delivery systems achieved mega successes due to their outstanding pharmaceutical properties and there have been several reviews on CDs based small molecule drug delivery systems recently. But for biomolecules, which are getting more and more important for modern therapies, however, there are very few reviews to systematically summarize and analyze the CDs-based macro biomolecules delivery systems, especially for proteins. In this review, there were some of notable examples were summarized for the macro biomolecules (proteins and NAs) delivery based on CDs. For proteins, this review included insulin, lysozyme, bovine serum albumin (BSA), green fluorescent protein (GFP) and IgG’s etc. deliveries in slow release, stimulating responsive release or targeting release manners. For NAs, this review summarized cationic CD-polymers and CD-cluster monomers as NAs carriers, notably, including the multi components targeting CD-based carriers and the virus-like RNA assembly method siRNA carriers.

Key words: cyclodextrins, drug delivery systems, proteins, nucleic acids, particles

Contents

1. Introduction

Proteins and NAs are the most vital fundamental biomolecules to support lives. The peptides are the fundamental structures of functional proteins that regulate thousands of bio-reactions in lives. The nucleic acids carry genetic information for lives and regulate bio-reactions as well in some proper ways. Therefore, both proteins and NAs can be employed as drugs in vast diseases especially in metabolic disorders, respectively.

The proteins based drugs achieved great successes in the last 3 decades, especial after the outcome of the representative diabetes protein drug—insulin and other protein drugs such as monoclonal antibodies, recombinant proteins, protein-based vaccines, etc., applied in almost every field of diseases such as cancer, inflammatory diseases, metabolic diseases and diagnostics[1–3]…In general, compared to the conventional small-molecule drugs, the protein drugs revealed great benefits such as higher specificity, greater activity and less toxicity[4]. The specific affinity and greater activity due to the proteins could selectively effect on signaling molecules that bind to cell surface receptors specific, like ion channels or G protein-coupled receptors, then induced the intercellular effects[5]. However, the protein drugs gained their own weaknesses such as large size or molecular weight, enzymatic degradation, poor permeability, fragile structure, easy aggregation, poor stability, elicit the over immunologic response, etc. All these weaknesses posed a mega challenge—how hard dose the protein drugs permeate to cells, tissues and organs!

Another challenge for protein is the deficiency of formulations, most proteins deeply relied on subcutaneous or intravenous injections, unlike the chemotherapy agents, most of them couldn't be prepared for oral formulations—the most common and easy acceptable administration to patients. For proteins, even there were and are a lot of investments in non-invasive delivery strategies for proteins, however, there were just very few successful examples[6,7]. As for oral formations of proteins studies, the reality is that the oral formulations were 10% as subcutaneous does in bioavailability evaluation in general[8,9]. Although there were some approved small proteins delivered via intranasal method[10], the large proteins seem not possible delivered by intranasal formulation. No matter what kinds of formulations it would eventually take for proteins, the fundamental requirement is an appropriate delivery system for proteins, which could overcome these weaknesses mentioned and improve the bioavailability as well.

Gene therapy holds great potential for the treatments of vast diseases including cancers[11], metabolic disorders[12], infections of microorganism[13], vaccines deponed diseases[14] and especially for inherited diseases[15], therefore, they are considered as the terminators of diseases.

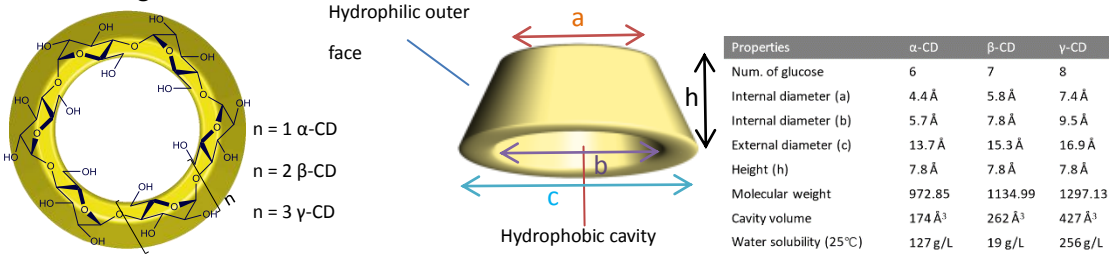
Typical gene therapies including plasmid DNAs, anti-sense oligonucleotides, small interfering RNAs, genetically engineered cellular therapies, etc., can be classified as DNA and RNA therapeutics. The DNA therapeutics exhibited great success in rare diseases' therapy, the first approved anti-sense oligonucleotides, *Vitravene*, for the cytomegalovirus (CMV) posed immunocompromised retinitis in the late 1990s, *Spinraza* for the spinal muscular atrophy (SMA), *Tegsedi* for the polyneuropathy of hATTR, etc.[16] Similar situation also occurred in the RNA therapeutics, such as the approved RNA drugs: *Eteplirsen* for the Duchenne muscular dystrophy; *Volanesorsen* for the familial chylomicronemia syndrome; *Inotersen* for the hereditary transthyretin amyloidosis, etc.[17]. The gene therapeutics also suggested a great potential in common diseases therapy. According to the Watts et al. summary, there are at least 431

RNA-targeting drug programs in different development stages and oligonucleotide companies were drastically increased 94.2% in the last 5 years. Also in the last 5 years, the private equities invested US$2.8 billion only on three representative mRNA therapeutic companies (Moderna Therapeutics, BioNtech, and CureVac)[17].

The key factor for the success of gene therapy is to avoid the biodegradation of gene-drugs in any status of the transfected process to the targeted cells. While, the nucleic acid's intrinsic properties prevent the transfection process generally, due to their large molecular size, polyanionic phosphodiester linkage and sensitive to nuclease[18,19]. Hence, like the protein drugs, the nucleic acids also need an appropriate delivery strategy to be transfected to targeted cells.

CDs as very successful delivery molecules can play vital roles in solving these macro biomolecules delivery issues, typical CDs are a family of basket-shape cyclic oligosaccharides, composing 6, 7 and 8 glucoses, named α-, β- and γ-CDs, respectively. Hydroxyls on 6-Cs called primary hydroxyls, on 2-Cs and 3-Cs called secondary hydroxyls, the hydroxyls on 2-Cs orienting to the inner cavity, and hydroxyls on 3-Cs orienting to the outer cavity[20]. Hydroxyls surrounded lead to the hydrophilic property on the outer of CDs, while the hydrophobic inner cavity attributed to the protons H-3 and 5 pointing to the inner cavity (see fig. 1). Due to amphiphilic character, almost nontoxicity, and cheap prices which CDs have long been employed in the food and pharmaceutical industry. In the beginning, the CDs employed as excipients in drugs' formation, and subsequently, as the observation of host-guest interaction (the hydrophobic groups at appropriate size can penetrate inside the CDs hydrophobic cavity which improves the poor water solubility), forming nanoparticles after being functionalized via self-assembly which, therefore, raised new opportunities for drug delivery to improve the bioavailability of poorly soluble drugs and achieved mega successes[21].

Though CDs applied in biomolecules delivery are not as common as in small molecules delivery, CDs also have a long history that employed in biomolecules delivery[22,23]. CDs as delivery carriers provided numerous of functionalization accesses which allowed desired functional groups introduced in, and the hydrophobic cavity also could form host-guest interaction with the hydrophobic groups of biomolecules that could improve their stability of the biofunction structures. More importantly, that it has been shown the CDs could strengthen the effects of proteins such as monoclonal antibodies, peptides and nucleotides[24]. As the importance of biomolecules rising, the more vital roles CDs will play, and however there just very few summaries of CDs applied in biomolecules delivery have been exhibited, that is, therefore, *cyclodextrins based delivery systems for macro biomolecules* has been prepared. More details of CDs based macro biomolecules' delivery features like the above described would be displayed in the following context.



Properties	α-CD	β-CD	γ-CD
Num. of glucose	6	7	8
Internal diameter (a)	4.4 Å	5.8 Å	7.4 Å
Internal diameter (b)	5.7 Å	7.8 Å	9.5 Å
External diameter (c)	13.7 Å	15.3 Å	16.9 Å
Height (h)	7.8 Å	7.8 Å	7.8 Å
Molecular weight	972.85	1134.99	1297.13
Cavity volume	174 Å^3	262 Å^3	427 Å^3
Water solubility (25℃)	127 g/L	19 g/L	256 g/L

Fig. 1. Structures, amphiphilic properties and their geometric parameters of CDs

2. The delivery of proteins

As the Canadian physiologist Charles Best, Sir Frederick Banting et al. first discovered that insulin was an effectual remedy to diabetes[25], the protein drugs continue emerging, till now it becomes a giant in the whole drugs market share. However, the intrinsic properties of protein such as large size, enzymatic degradation, short circulation half-lives, fragile tertiary structures and poor membrane permeability, etc. resulting in a huge barrier for their clinical applications[2,4,26]. Hence, appropriate delivery strategies could either overcome the above disadvantages or render targeting abilities.

Generally, researchers usually investigated their studies of CDs based protein delivery systems in protein loading experiments, delivery materials biodegrade experiments, cytotoxicity experiments, protein releasing experiments, etc. If there were nanoparticles of delivery systems had formed, a lot of nanoparticle parameters also had been studied, such as size, ζ potential, etc.

In this section the protein delivery methods would be introduced in terms of loading materials and followed the below orders: polypseudorotaxanes, supramolecular polymers, mutilate components targetable delivery system and functionalized CD monomers or dimers, displayed in table 1.

Table 1. The summary of proteins delivery and release methods

Loading materials	Proteins	Releasing methods	References	Years
CD-based polypseudorotaxanes	Insulin	Slow releasing	[27]	2008
CD-based polypseudorotaxanes	Insulin	Slow releasing	[28]	2009
CD-based polypseudorotaxanes	Lysozyme	Slow releasing	[29]	2009
CD-based polypseudorotaxanes	IgG, antibody, bromelain	Slow releasing	[30,31]	2015, 2017
CD-based polypseudorotaxanes	Insulin	Thermo responsive stimulating releasing	[32,33]	2017, 2020
Supramolecular polymers	BSA, IgG, lysozyme	Slow releasing	[34]	2008
Supramolecular polymers	Lysozyme	Slow releasing	[35]	2006
Supramolecular polymers	GFP	Photo responsive stimulating releasing	[36]	2006
Supramolecular polymers	BSA	Photo responsive stimulating releasing	[37]	2015
Targetable delivery systems	BSA, saporin, nuclease Cas9 protein	Targeted releasing	[38]	2019
Targetable delivery systems	DNAzyme	Targeted releasing	[39]	2019
Targetable delivery systems	Tyrosinase-related protein 2	Targeted releasing	[40]	2020
Functionalized CD	Insulin	Slow releasing	[41–43]	2011

monomers and dimers				
Functionalized CD monomers and dimers	BSA	Slow releasing	[44–46]	2005, 2006, 2007
Functionalized CD monomers and dimers	Lysozyme	Slow releasing	[47]	2007
Functionalized CD monomers and dimers	Bovine pancreatic trypsin	–	[48]	2004
Functionalized CD monomers and dimers	Insulin	–	[49]	2014
Functionalized CD monomers and dimers	α-Chymotrypsin	Slow releasing	[50]	2006
Functionalized CD monomers and dimers	Antibody	–	[51]	2014
"–" means the authors didn't mentioned in the cited articles				

2.1 Slow released proteins via CD-based polypseudorotaxane

Slow release insulin administration is very helpful to insulin-depend diabetes patients, it can minimize the insulin using numbers that can reduce the pain to patients marked. Uekama team reported PEGylated insulin/CD polypseudorotaxane controlled release systems for insulin[27]. Insulin has 3 free primary amino groups, one located on A chain, another two located on B chain of insulin which rendered multiply-PEGylated access sites to insulin. They coupled insulin with α-succinimidyl-oxysuccinyl-ω-methoxy-polyoxyethylene to form PEGylated insulin[52], then, assembled pseudorotaxanes with α- and γ-CDs (the PEGylated insulin couldn't form gel with β-CD) to form gels. *In vitro*, insulin release studies exhibited that both α- and γ-CDs polypseudorotaxanes could prolong the releasing time of insulin compared to PEGylated insulin alone. When some free α- and γ-CDs were added to the buffer that would further decrease the release rate of insulin than just CDs polypseudorotaxanes, because the threading and dethreading of polypseudorotaxanes processes were dynamic equilibrating. *In vivo* release study, the γ-CD polypseudorotaxane also showed a prolonged release manner than the PEGylated insulin, which could keep plasma glucose at low level in long time[27]. (See fig. 2)

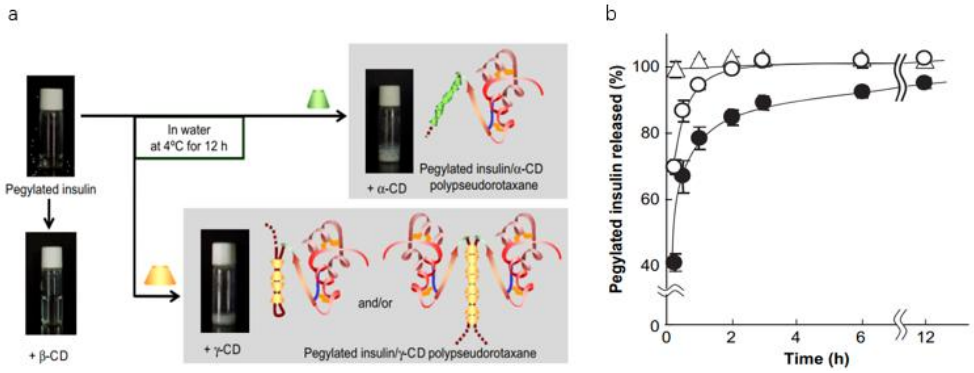


Fig. 2. a) PEGylated insulin assembled polypseudorotaxanes with α- and γ-CDs while can't form polypseudorotaxanes with β-CD, b) the release profiles of PEGylated insulin alone (Δ)or formed polypseudorotaxanes with γ-CD (o) and α-CD(•)[27]

In the following study, Uekama team devised randomly- or multiply-PEGylated insulins then

assembled polypseudorotaxanes with α- and γ-CDs for insulin delivery[28]. The PEGylation procedures followed the report of Kim et al. in selectively introduced PEG to primary amines[53,54]. That method in resulting, to give 35% of mono-substituted, 49% of di-substituted and 9% of tri-substituted PEGylated insulins according to the HPLC spectra. The PEGylated insulins and CDs were assembled to polypseudorotaxanes, followed the same procedure as the above study[27]. The insulin release trials indicated that the polypseudorotanxanes were remarkably decreased in release rate than PEGylated insulin in general. Compared the insulin release rate of α- and γ-CD polypseudorotaxanes, the α-CD polypseudorotaxanes displayed much slower release rate, in addition, the release rate were largely related it's concentration in phosphate buffer, for instance, the same mounts of insulin-α- and -γ-CDs polypseudorotaxanes in 1 mL, 0.85 mL and 0.45 mL of phosphate buffer displayed different release rates (the rate: 1 mL > 0.85 mL > 0.45 mL). The equilibrated threading and dethreading of polypseudorotaxanes processes probably could explain those slow release results[28].

Inspired by the above study, Uekama et al. investigated a twice larger protein lysozyme's delivery. A similar PEGylation strategy was employed on lysozyme's delivery, PEGylated lysozyme assembled polypseudorotaxanes with α- and γ-CDs. The release study suggested the release order was PEGylated lysozyme > α-CD polypseudorotaxane > γ-CD polypseudorotaxane[29]. From insulin to lysozyme, it indicated that the CDs-PEG polypseudorotaxane systems could be used for different proteins' delivery that provided a slow release model to different proteins or other molecules delivery. (See fig. 3)

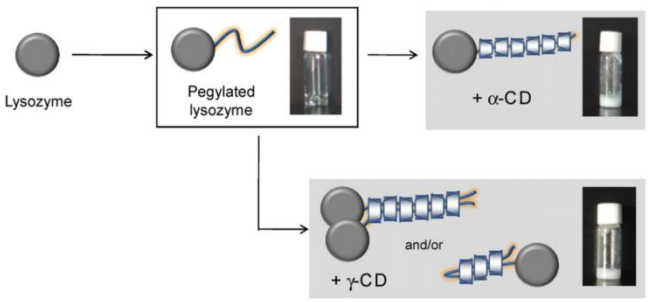


Fig. 3. PEGylated lysozyme assembled polypseudorotaxanes with α- and γ-CDs[29]

However, in terms of some study, that the PEGylation of insulin or lysozyme could reduce their bioactivities, due to the steric hindrance from large PEG chains or PEGylation originated structure transformation of proteins, for insulin the activity reduced to less than 6%, and for lysozyme, the number was 70%[24]. (See fig. 4a) Therefore, the Arima team's resolution for this problem was applied an ignorable small molecule—adamantane (Ad) to conjugate to insulin, and then the PEGylation occurred on CDs, which subsequently, formed host-guest complexes with Ad-insulin that could retain the activity of insulin after release, this had been called "self-assembly PEGylation retaining technology" (SPRA) by authors[55]. (See fig. 4b)

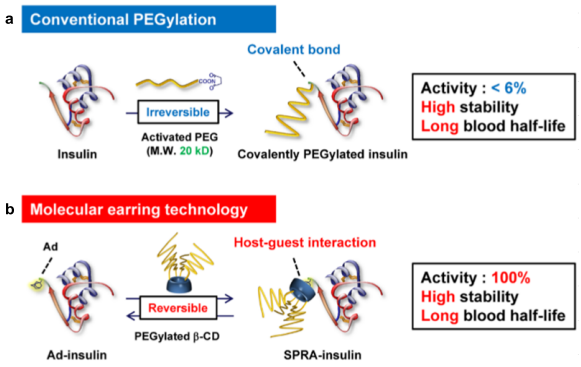


Fig. 4. Activity of PEG-insulin and Ad-insulin assembled with PEGylated-CDs[24]

Uekama and Hirayama team also developed a thermo-responsive injectable sustained release drug delivery system based on CD/hydrophobically modified hydroxypropylmethylcelluse (HM-HPMC) formed hydrogel[32,33]. The working mechanism was due to the HM-HPMC/CD hydrogel with a low viscosity at low temperatures, therefore, the hydrogels were easy to inject, while, at a high temperature like 37°C, the gels were in high viscous status, that resulted a sustained molecule release manner. The hydrogels assembled via host-guest interactions by free CDs and HM-HPMC to form polyrotaxanes (see fig. 5a). The sustained-release effect of the HM-HPMC/CD hydrogels was investigated as following, the model drug insulin was loaded then injected to the rats, the free insulin was also injected to another group as comparability and the PBS was injected to the 3rd rat group as blank control. As the conclusion, the plasma glucose level tested results showed the HM-HPMC/CD/insulin injection group kept at 40 mg/dL for 6 h while for free insulin injection group was 4 h and increased to 80 mg/dL very fast in 2 h[33] (see fig. 5b). In terms of the release study results and the working mechanism, the thermo-responsive strategy displayed great potential in sustained-release drug delivery not just including protein drugs.

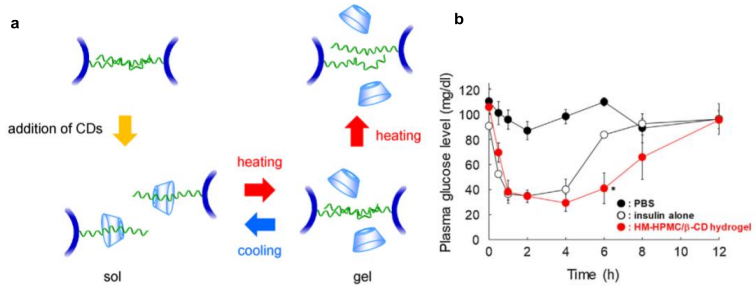


Fig. 5. a) Thermo-responsive HM-HPMC/CD gel working mechanism[32], b) HM-HPMC/CD delivered insulin exhibited marked plasma glucose control manner[33]

2.2 Supramolecular interaction-based bio-degradable and stimulating responsive release protein carriers

Hennink team reported a bio-degradable—PEG-β-CD/PEG-cholesterol—hydrogel applied for proteins delivery. They designed and prepared 8-arm PEG with different molecular weights (10, 20 and 40 kDa) grafted β-CD or cholesterol, then, formed gel via self-assembling (see fig. 6)[34]. They employed 3 different proteins lysozyme, BSA and IgG to evaluate the release profiles of the mentioned hydrogels. The 3 proteins were loaded on 22.5% (w/w) $PEG_8$20K-β-CD/$PEG_8$20K-chol (PEG_8 represents 8-arm PEG, 20K represents its mole weight grade, chol represents cholesterol) gel, respectively, then under the release conditions[56] to determine their release rates. The

results exhibited a striking sustained release manner that lasted more than 9 days. The authors attributed this astonished slow release effect to the slower surface erosion, in addition, when the proteins released the hydrogels were degraded which was perfect for the clinical application[57].

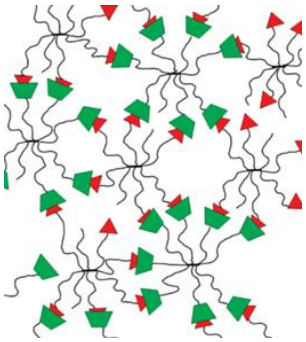


Fig.6. PEG-β-CD/PEG-cholesterol hydrogel[34]

Stimulating responsive controlled release proteins delivery is always sought by modern pharmaceutics, the noncovalent interactions like supramolecular interaction provide a perfect option for the stimulating responsive manner for its reversible interaction. Kros team reported a cyclodextrin based photo-switchable crosslinked hydrogel applied on protein delivery[58]. The components of this hydrogel consisted a "guest chain"–maleimide functionalized dextran (Mal-Dex), on which an azobenzene grafted via the thiol-maleimide "click" reaction[59], and a "host chain"–β-CD grafted on another maleimide functionalized dextran (CD-Dex), also via the thiol-maleimide "click" reaction (see fig. 7a). To evaluate the stimulating release property of this gel, GFP as model protein was loaded. Under UV irradiation (λ = 365 nm), the azobenzene transformed to cis isomer, dissociated inclusion interaction, caused the dissolution of the hydrogel[36], then added GFP, next removed the UV irradiation, the GFP was physically entrapped due to the azobenzene transformed to trans, formed inclusion interaction again. The release study exhibited when the hydrogel under UV irradiation (λ = 365 nm), the GFP release improved markedly in a stimulating responsive release manner (see fig. 7b).

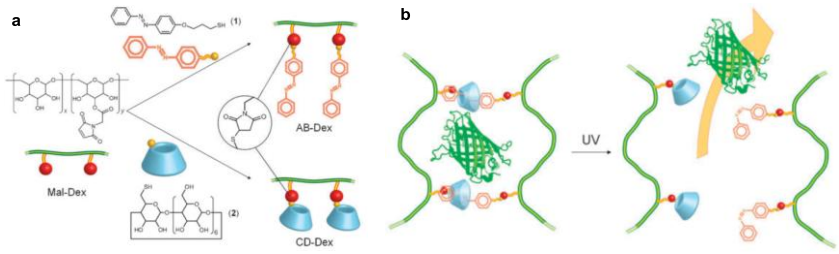


Fig. 7 a) components of supramolecular interaction based light responsive protein carriers, b) the protein released after UV irradiation[58]

Based on similar supramolecular interaction strategy, Wu team[37] applied tetra-*ortho*-methoxy-substituted azobenzene (mAzo) and β-CD as host-guest interaction components both grafted on backbone poly(acrylic acid). When the system irradiated by red light (600-900 nm), the mAzo transformed to trans isomer, allowed the system to form host-guest complex (gel form) at the same time encapsulated protein (BSA), however, when the system irradiated with blue light, the mAzo transformed to *cis* isomer, the host-guest complex broken to give solution form, allowed the system to release proteins. (See fig. 8)

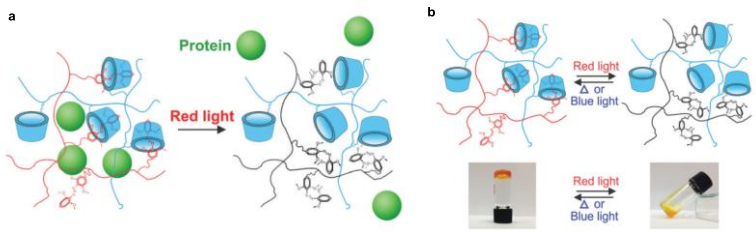


Fig. 8. a) Proteins were loaded under gel form (host-guest interaction), and released under solution form after red light irradiation, b) the gel and solution form could transform under different lights irradiation[37]

2.3 Targetable protein vectors based on CDs

CD-based targeting proteins delivery also attempted by scientists, Feng team developed a targeted intracellular protein delivery system based on functionalized β-CD called CDEH by the authors[38]. The CDEH is made of the moieties of selective conjugated hydrophobic chain via ionic linker to CD on the primary face. They chose BSA as model protein to evaluate the CDEH's protein loading capacity, the results revealed that the mass ratio of CDEH and BSA reached 2:1, the BSA could be entrapped completely. Under the same ratio of CDEH and saporin, the saporin which has been used for cancer therapy in clinical trials, could also almost be entrapped completely. MDA-MB-231 cells were selected for *vitro* cellular uptake study, MDA-MB-231 cell membranes overexpressed nucleolin receptors which could bind to AS1411 aptamer (AP)[60]. Then the AP as recognition molecule to MDA-MB-231 was introduced through the conjugation to adamantane, which allowed the AP physically grafted on CDEH via the host-guest interaction. (See fig. 9) The authors compared the free saporin, CDEH/saporin and targeted CDEH-AP/saporin in their cellular uptake efficiency suggested that the free saporin didn't cause any cell death due to its poor cell membrane permeability, CDEH/ saporin exhibited significant higher cell growth inhabitation, and CDEH-AP/saporin showed the best inhabitation.

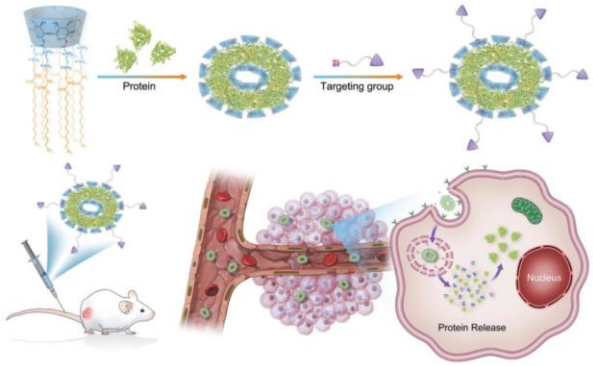


Fig. 9. Saporin loaded on targetable CDEH to form nanocomplexes and their uptake mechanism on mice[38]

The authors also applied this system on the delivery of nuclease Cas9 protein and sgRNA (small guide RNA), which both are employed in the prevalence genome editing tool today, called CRISPR/Cas9 genome editing system[61,62]. In this system, the sgRNA can specifically recognize the complementary DNA and guide the Cas9 protein to cleave the target DNA sequences precisely and then edit the genome successfully[63]. Nevertheless, how to deliver the Cas9 protein and sgRNA remains a real challenge nowadays, therefore, the authors employed the modified CDEH nanoparticles for the Cas9 protein loading. Polo-like kinase 1 (PLK1), usually over expressed on many tumor cells and which had been inhibited that could induce tumor cell

apoptosis. Therefore, PLK1 targeted sgRNAs were co-loaded with Cas9 protein on CDEH then treated the Hela cells resulted in 47.1% cleavage of the target gene and rendered the proliferation inhabitation significant.[38] In terms of the above results, the CD-based CDEH delivery system holds great potential on multi-functions, it could be applied as protein or protein/RNA delivery which provided a fresh new option for genome editing.

Chemotherapy is still one of the most important cancer treatments nowadays, while the resistance of anticancer agents is largely reducing the chemotherapy efficiency somehow. P-glycoprotein 170 encoded via multi-drug resistance 1 gene (MDR1) over expressed plays a key role in the drug resistance[64]. The Nematollahi-Mahani team[39] reported a downregulating the MDR1 strategy to reduce the P-glycoprotein 170 expressing, subsequently, decreased the drug-resistance effect. The mRNA-cleaving DNAzyme (DNZ) can regulate the mRNA expressing was implicated by numbers of evidences[65], therefore, the authors chose a mRNA-cleaving DNZ which could target the mRNA of MDR1 in doxorubicin (Dox) resistance breast cancer, further downregulated the P-glycoprotein expressing resulted in reducing the drug resistance effect. The authors applied the reported chitosan cross-linked β-CD and pentasodium tripolyphosphate (TPP) as the delivery vector for DNZ[66]. The WST1 study results revealed that Dox associated with DNZ-MDR1 could induce cell death in the drug resistance cells significantly compared to 5 μg/mL DNZ-unspecific treated MCF-7 cells. The MDR1 mRNA and P-glycoprotein 170 expression reduced results also cross-validated the above results[39].

The antitumor protein vaccines hold great potential in tumor therapy which successfully attracted a lot of attention from scientists. Before the vaccine proteins working as vaccines, one mechanism is that the protein should be uptake by dendritic cells (DCs). Enhanced the cellular uptake efficiency of DCs, triggers a strong immune efficacy but avoided toxicity over the non-specific effects at the same time. In order to improve the cellular uptake efficiency, Hu team developed a CD-based protein co-delivery system for both melanoma antigen protein Tyrosinase-related protein 2 (Trp2) and Toll-like-receptor-7 (TLR-7) agonists imiquimod (R837)[40]. Trp2, a confirmed melanoma tumor-associated antigen[67] and R837, a robust vaccine adjuvant could promote immune efficacy[68] were chosen to combine as a vaccine in Hu team's devise. CD provided a hydrophobic cavity for the R837 and the mannose grafted on CD could specifically interact with the DCs, on which surface abundantly expressed mannose receptors. TAT, a cell-penetrating peptide (CPP), was employed to improve the solubility of Trp2 via a conjugation between TAT and Trp2 to give a product named WT[69] that would increase the cellular uptake ability to WT by DCs. Subsequently, the co-delivery nanocomplex system was assembled Man-CD/R837, sodium alginate (SA) and WT via electrostatic interaction. (See fig. 10)

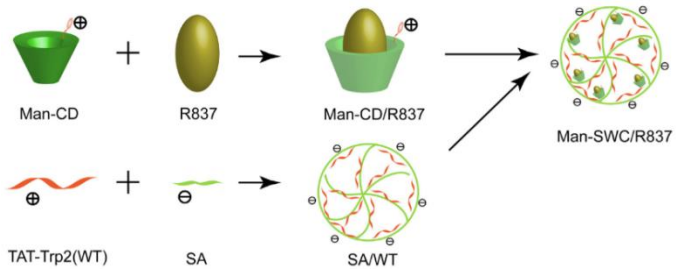


Fig. 10. Preparation of mSWC/R837[40]

The authors prepared two kinds of nanocomplexes, Man-CD/R837, CD/R837 and SA/WT, called

mSWC/R837 and SWC/R837, respectively. The cytokines secretion determination study results displayed that the nanocomplex delivered vaccine induced highest level of cytokines secretion compared to WT alone or SWC/R837, especially IFN-γ secretion in BMDCs. And also the mSWC/R837 also induced a higher level of IgG in serum than WT alone, mSWC alone and SWC/R837 in day 7, 21 and 28 which indicated that the mSWC/R837 induced greater lymphocyte activation effector cytokine secretion and led to a synergistic cellular immune response effect that endowed this co-delivery system for vaccines great potential in clinical applications[40]. (See fig. 11) Additionally, an earlier study in 2016 was investigated by Ishii team[70], which also proved that the hydroxypropyl-β-CD employed as adjuvant of vaccine could also stimulate a serum IgG response via intranasal administration.

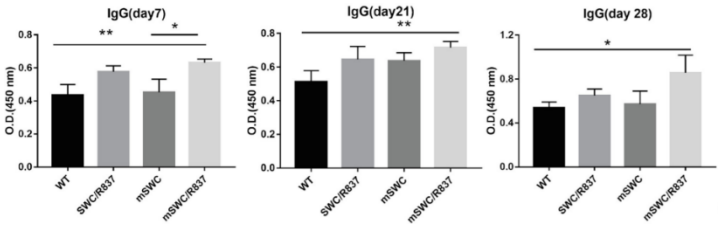


Fig. 11. WT, SWC/R837, mSWC and mSWC/R837 induced IgG secretion level in 7, 21 and 28 days[40]

2.4 CD-functionalized monomers or dimers applied as protein delivery vectors

Except the CDs-PEG polypseudorotaxane insulin delivery systems, Uekama team also developed sulfobutyl ether-β-CD (SBE-β-CD) and maltosyl-β-CD (G_2-β-CD) as insulin delivery systems. The authors discovered that the SBE7-β-CD and G_2-β-CD could influence the aggregation of insulin, even not in the same mechanism[71] but all of them could enhance the bioavailability and persistence, and applied in insulin administration could prolong the release process marked, compared to insulin glargine (see fig. 12a and 12b). The authors proposed this outcome to the inhabitation of SBE7-β-CD on the enzymatic degradation of insulin[41,43]. Notably, the SBE7-β-CD and G_2-β-CD/insulin administrations exhibited peak-less sustained hypoglycemic effect compared to insulin glargine[42,72].

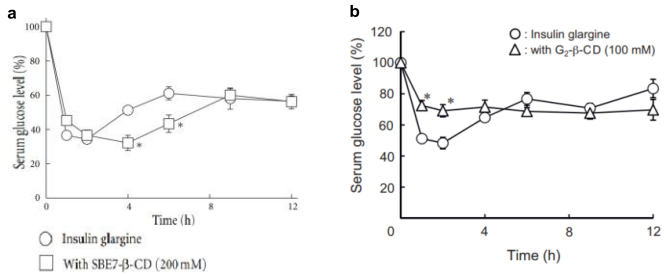
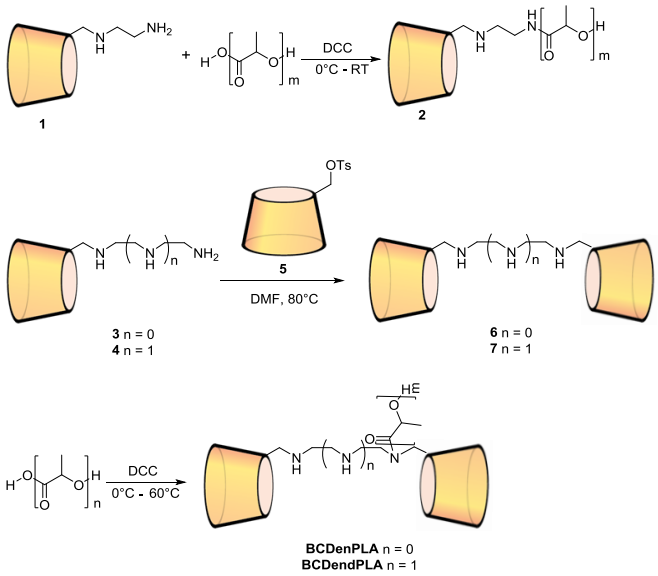


Fig. 12. Both SBE7-β-CD and G2-β-CD loaded insulin displayed comparative sustained hypolycemic effect compared to indulin glargine and peak-less effect as well[42,72]

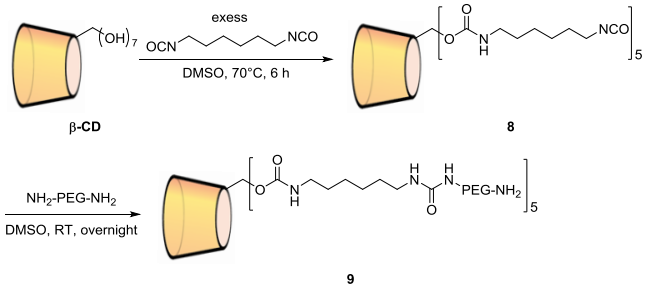
Ma team[44] reported a copolymer, consisted of the poly(dl-lactide) (PLA) and β-CD via the linkage of ethylenediamine, CDenPLA (**2**) which could fabricate nanoparticles with protein, subsequently, to fulfill the purpose of protein delivery. They compared the loading capacity of bovine serum albumin (BSA) with encapsulation efficiency (EE%) between CDenPLA and its conjugated PLA alone, found out that the EE% of CDenPLA polymers is much higher than PLA

polymers. In addition, different nanoparticle fabrication methods[73,74] showed different loading capacities. And the BSA release profiles of CDenPLA were faster compared to its conjugated PLA alone but both releasing processes could sustain to a mouth or more[44,46]. Then they figured out that the bisaminated β-CDs were easier to form a complex with BSA than the monoaminated β-CD[75], hence, β-CD dimer was grafted on PLA to form so called BCDenPLA and BCDendPLA delivery systems (see scheme 1). The BCDenPLA and BCDendPLA system in model protein BSA's delivery exhibited good loading capacity while related to its particle size, for instance, 300-400 nm in diameter particles' EE reached 70-83%, in contrast, sized 150-250 nm particles' EE was 40-50%. In addition, the BCDenPLA and BCDendPLA systems revealed a BSA slow release behavior that could last 30 days as well[45].



Scheme 1. Synthesis of CDenPLA (**2**), BCDenPLA and BCDendPLA

CDs and PEG both hold remarkable biocompatibility and hydrophilicity characteristics in drug delivery, hence, the Caliceti team devised a series of CD/PEG hydrogels consisted different CD/PEG molar ratios, to deliver proteins like lysozyme[47]. They applied β-CD to link with NH_2-PEG-NH_2 via hexamethylene diisocyanate, obtained CD-PEG conjugates[76,77]. (See scheme 2)



Scheme 2. Synthesis of CD-PEG conjugates

The lysozyme loading study suggested that when the CD/PEG ratio went higher, the loading capacity decreased while the release rates were similar in different CD/PEG components. To explain that the author attributed the CD/PEG hydrogel formed tight networks as a major factor which prevented the lysozyme penetrated in hydrogels[47].

To improve the cell-penetration ability, Huang team inspired by the cell-penetrating peptides (CPPs) as penetratin (PEN) used for biomolecules delivery[78], especially insulin delivery[79], therefore, developed a PEN conjugate to the bis-β-CD delivery system for insulin[49]. Generally, the CPPs were considered as nontoxic in their working concentration[80] so as CDs. The CPPs could interact with the insulin charged groups via electrostatic effects, while the CDs could include some insulin hydrophobic groups formed complex[81]. Therefore, the PEN-bis-CD (P-bis-CD) could form a complex with insulin both through electrostatic and hydrophobic interactions following led nanocomplexes assembly.

The authors then assembled two nanocomplexes employed insulin with PEN directly and insulin with P-bis-CD (see fig. 13). After the complex stability, enzymatic stability and cellular uptake studies, the P-bis-CD displayed better results in all parameters than PEN, and much better than free insulin[49]. This design successfully combined both PEN and CD's advantages and significantly improved insulin deliver efficiency.

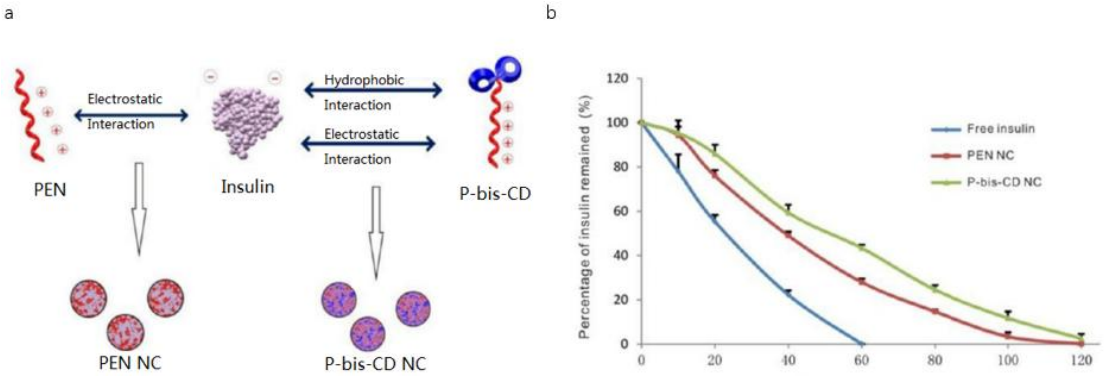


Fig. 13. a) Preparation of PEN/insulin nanocomplexes and P-bis-CD/insulin nanocomplexes, b) the stability of different loaded insulins over trypsin[49]

3. The delivery of DNA and RNA

Since the biophysicist Crick and biochemist Watson developed the revolutionary model of the double-strand DNA in the 1950s, these magic molecules have been attracted so much attention from not only the scientists but also the public.[82] As people continuously studying in genetic science, they found out that almost every disease could be traced to genes except some physical injured. Therefore, gene therapy is a promising strategy, that may allow doctors to treat diseases by inserting a certain gene into patients' cells instead of utilizing drugs or surgeries, which can be the final solution for vast diseases. But how to deliver the nucleic acids (NAs) safely and efficiently into patients' cells is still a mega challenge to us by now. Basically, there are two types of major transferred techniques, the viral and non-viral vectors. As for the viral vector, it possessed carrying capacity, high cost, immunogenic response, toxicity and oncogenicity issues.[83–85] Therefore, the liposome based non-viral vector attracted a lot of attention and became an alternative for it can avoid most viral vector's disadvantages.[86] However, the non-viral based carrier has its own limitation such as toxicity (such as high density charge of poly-ethyleneimines (PEI))[87] as well, and even low efficiency and poor selectivity compared to the viral one.[88] To get across the above shortcomings of the non-viral vector, CDs came out as an attractive alternative tool for the scientists to operate, for its appropriate properties such as good water solubility, easy functionalization accessible and non-toxicity.

Generally, CDs-based NAs delivery systems, researchers usually investigated their studies on Ns

loading experiments, delivery materials biodegrade experiments, cytotoxicity experiments, NAs transfection and releasing experiments, etc. if there were nanoparticles formed, a lot of nanoparticles parameters also been studied, such as size, ζ potential, etc.

In this section the NAs delivery methods would be introduced in terms of loading materials and will be described as the below orders: polypseudorotaxanes, supramolecular polymers, mutilate components targetable delivery system and functionalized CD monomers or dimers, displayed in table 2.

Table 2. The summary of NAs delivery methods

Loading materials	NAs	References	Years
Cationic polymers	pDNA	[89–92]	1999, 2001, 2003, 2004
Cationic polymers	siRNA	[93]	2013
Cationic polymers	siRNA	[94]	2011
Cationic cluster monomers	pDNA	[95]	2008
Cationic cluster monomers	pDNA	[96–100]	2009, 2011, 2013
Cationic cluster monomers	pDNA	[101,102]	2004, 2011
Cationic polyrotaxanes	pDNA	[103,104]	2007, 2009
Cationic polyrotaxanes	pDNA	[105]	2012
Cationic polyrotaxanes	pDNA	[106]	2008
Cationic polyrotaxanes	pDNA	[107]	2006
Cationic polyrotaxanes	pDNA, siRNA	[108,109]	2012, 2017
Cationic polyrotaxanes	siRNA	[110]	2013
Cationic polyrotaxanes	siRNA	[111]	2012
Targetable cationic polymers	pDNA	[112]	2011
Targetable cationic polymers	pDNA	[113–116]	2004, 2007, 2009, 2011
Targetable cationic polymers	pDNA, siRNA	[117,118]	2016
Targetable cationic polymers	siRNA	[119]	2017
Monomers assembled supramolecular materials	siRNA	[120]	2018

3.1 DNAs' delivery

3.1.1 Cationic polymers applied as DNA delivery vectors

Davis and co-works devised and prepared a series of linear cationic (because the linear of NAs shown negative charges, which can coordinate to the cations) β-cyclodextrins (CDs)-based effective and low toxic gene delivery vectors. They introduced the ethanethioamine as the charge centers on the primary rim of β-CD to form monomer, 6^{A},-6^{D}-dideoxy-6^{A},-6^{D}-di(2-aminoethanethio)-β-CD. Subsequently, they cross-linked the monomer

over the DMS to obtain the β-CD contained polymers (molecular weight 8800) in 24% of overall yield. The polymer and plasmid DNA (pDNA) binding experiments resulted the charge ratio was 1:1.5 which exhibited that the polymer was able to bind the pDNA completely. The *vitro* transfection and toxicity of DNA complexes assay show that 1) the Luciferase protein activity in BHK-21 cells transfected in serum-free conditions reached a stable maximum at 30+/- with ~1×10^9 RLU/mg of protein; 2) the toxicity was minimized with the presence of 10% serum during transfection. The results approved the polymers containing β-CD as gene delivery cargo with satisfied the effective and low toxic criteria[89]. To better understand the length of the spacer, the authors varied the length of the spacers then they found that the toxicity was increasing as the length increases in going from β-CDP4 to β-CD8.[90](See fig. 14)

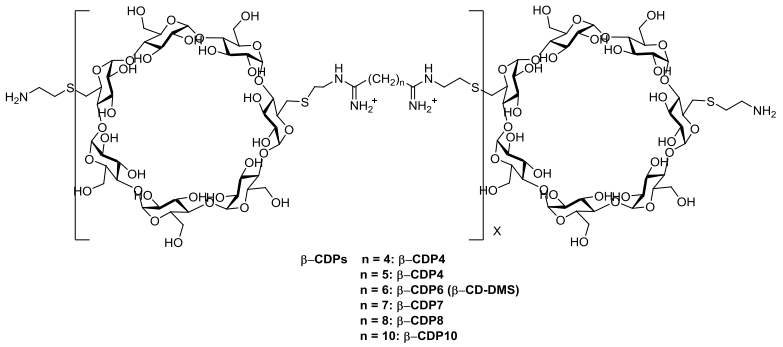
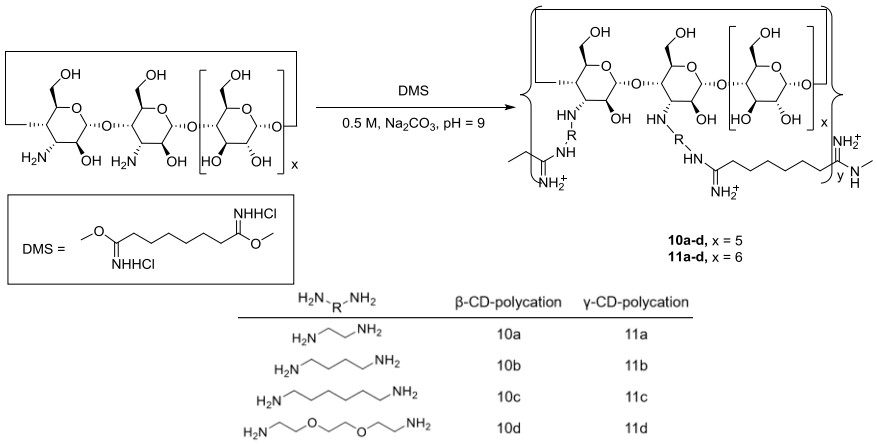


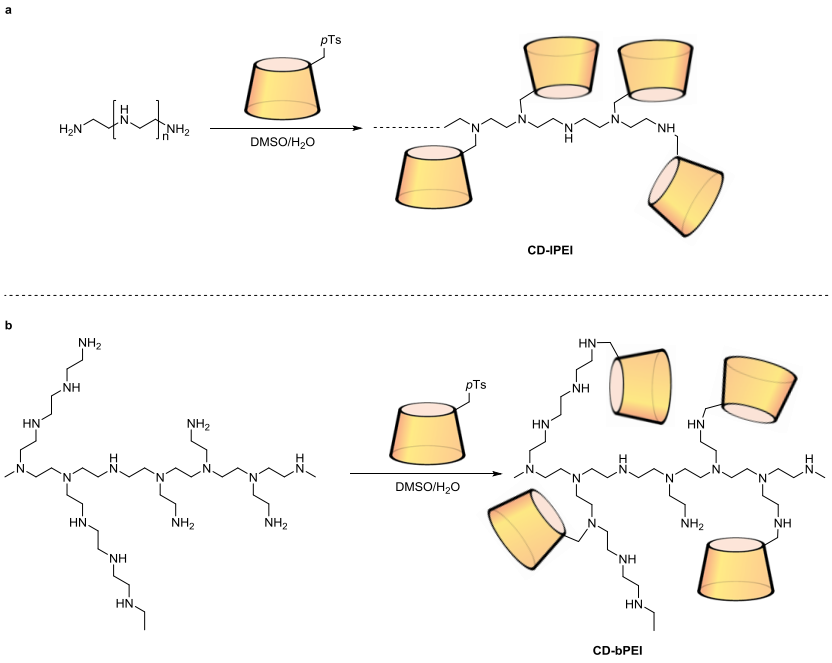
Fig. 14. Different spacer lengths of CD polymers

Inspired by their previous work, Davis and co-works introduced aminoalkylamine and aminoalkoxyamine as pedals on the secondary rim of β- or γ-CD then conjugated with the DMS as the charge center to form $3^A,3^B$-di(aminoalkylamino)-β-cyclodextrin and $3^A,3^B$di(aminoalkoxyamino)-γ-cyclodextrins (**10a-d** and **11a-d**). (See scheme 3) The toxicity assay demonstrated that the polycations **10a-c** and **11a-c** in cell viability with a pronounced decreased as the spacers prolonged while, **10d** and **11d** were non-toxicity at all concentrations employed by authors. In addition, when increased the charge ratio of polycation and pDNA, the cell viability decreased for both **b** and **c** analogues, but for the β-CD was worse compared to γ-CD. As for the transfection efficiency, the polycations, **10a-c** and **11a-c**, prolonged the spacers, produced a better transfection efficiency.[91] In general, the introduced CDs could increase the cell viability and the NAs transfection efficiency apparently.



Scheme 3. Synthesis of linear DMS linked CD polymers

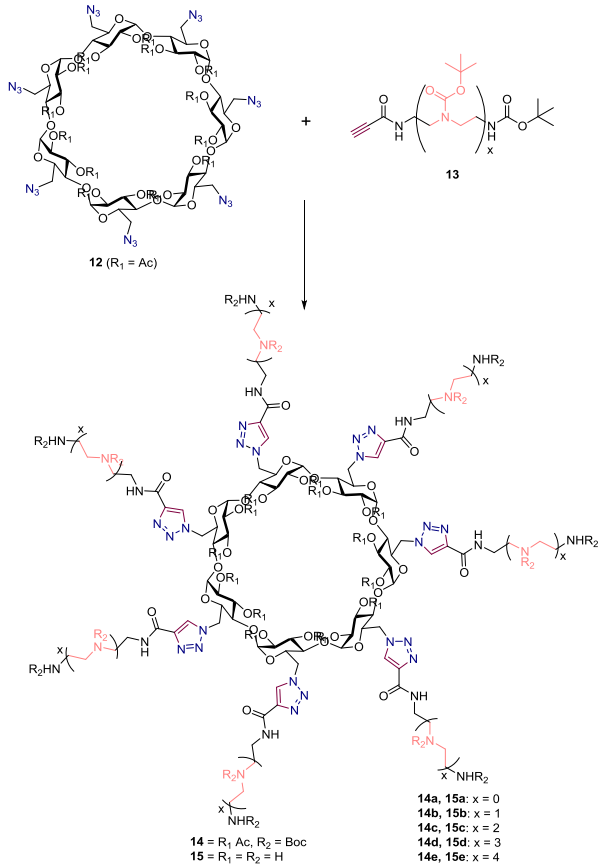
Poly(ethylenimines) (PEI) can provide efficient gene transferring *in vitro*[121], nevertheless, the toxicity and difficulty in formulation of PEI is limiting its application in gene delivery. The LD_{50} of linear PEI (lPEI) were approximately 4 mg/kg (Balb/C mice)[122], while the CD-based polymers were 200 mg/kg (Balb/C mice)[90]. To combine both benefits of PEI and CD polymers and minimize the toxicity of PEI, Davis[92] team designed and synthesized the β-CD grafted PEI (CD-PEI) polymers applied non-viral gene delivery. They employed both the linear and branched PEI (lPEI and bPEI) conjugated with β-CD to prepare the CD-PEI polymers (see scheme 4a and 4b), then, added adamantine-poly ethylene glycol (AD-PEG) to the polymers to increase the stability and formulation ability of CD-PEI polymers. In the transfection and toxicity assays in PC3 cells, displayed in 3 general outcomes, 1) reduced the toxicity acutely, 2) stabilized the vectors in physiologic salt solutions, 3) good transfection efficiency, as the authors anticipated.



Scheme 4. Synthesis of bPEI grafted CDs (a) and lPEI grafted CDs polymers

3.1.2 Cationic cluster monomers applied as DNA delivery vectors

Reineke[95] and co-workers described a series of multivalent clusters based on β-CD. They introduced 7 azide groups on the primary rim, then, conjugated the different alkyne dendrons via the “click” reaction to form motifs **15a-e** (see scheme 5). The transfection and toxicity assays showed that all the compounds **15a-e** were minimal cytotoxicity, the **15d** gave the best transfection efficiency of all compounds nevertheless, **15b** indicated moderate efficiency, in HeLa cell lines. To explain that the **15d** exhibited the best transfection efficiency compared to other derivatives, the authors considered that for example the **15b** bond the pDNA weak and hence lack of pDNA protection against enzymatic degradation. On the opposite, a much stronger combination of **15d** and pDNA, led to a stronger protection of pDNA from enzymatic degradation, consequently, a higher gene expression.



Scheme 5. Synthesis of CD cluster via a "click" reaction

In 2006, García Fernández and co-workers[123] reported that the urea, thiourea and guanidinium could form hydrogen bond with phosphate via their dual amino groups that made the dual amine structure became the binder of phosphate which are the fundamental units of nucleosides. (See fig. 15) Subsequently, therefore, García Fernández group devised monodisperse facial amphiphiles consisting of a β-CD-based multivalent polycationic groups (aminoethyl amine and aminoethyl thiourea) at the primary/secondary face and grafted hydrophobic chains at the secondary/primary face clusters as shown in figure 16 (a and b) to deliver the NAs[99,124] They toke the similar strategy to form CD monomers **16-19**. All these monomers could form polyplex with pDNA exhibited significantly transfection efficiency under the absence of serum condition.[96,99,124] Nevertheless, only **17** still revealed transfection efficiency with the presence of serum which was vital for the *vivo* trials.[100] In terms of the authors' description, the preparation of **17**could be the handicap by its high cost, therefore an easy operable and cheaper approach was expected. Looked back on the CD monomers **16-19**, the authors figured out that the "click" strategy to obtain monomer **19** was a reasonable access to the CD cluster. To improve the "click" method they employed solid-supported Cu(I) catalysts, thereby simplifying the purification procedure. A series of triazole and thiourea products were prepared, and then the prepared chemicals **20**-**22**, **23**, **24**, and **25** can fully co-assemble with pDNA to form nanoparticles. (See fig. 17) The transfection and toxicity trials revealed that, **20** had better transfection results than **17** under the 10% serum presence, probably because the longer hydrophobic chains. While if the length of the hydrophobic chains were increased too much to generate **21** and **22**, that would weaken the transfection ability. As for the trizole-thiourea CDs,

the derivative **25** shown better transfection performance than **16**, **17**, **18**, **19** or **23** in the absence of serum and lower cytotoxicity but worse transfection than just **17** in the presence of 10 % serum.[98]

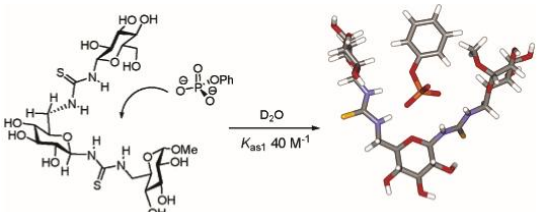


Fig. 15. Phosphate could bind with dual thiourea[123]

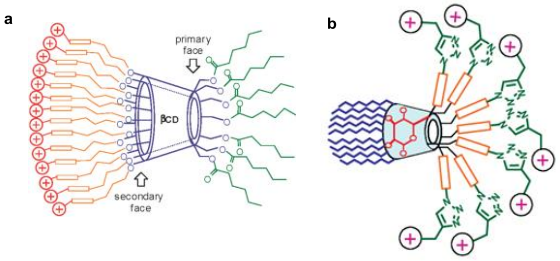


Fig. 16. García Fernández team designed and prepared monomer with cation groups on secondary face and hydrophobic groups on primary face of CD (a)[124] or cation groups on primary face and hydrophobic groups on secondary face of CD (b)[99]

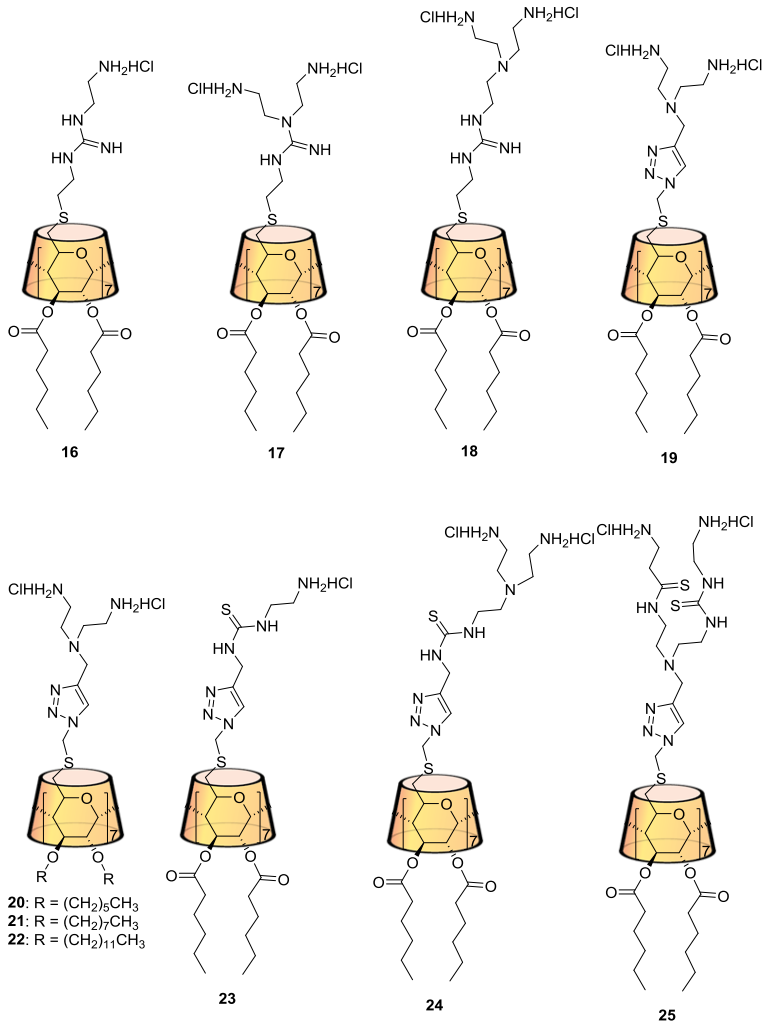
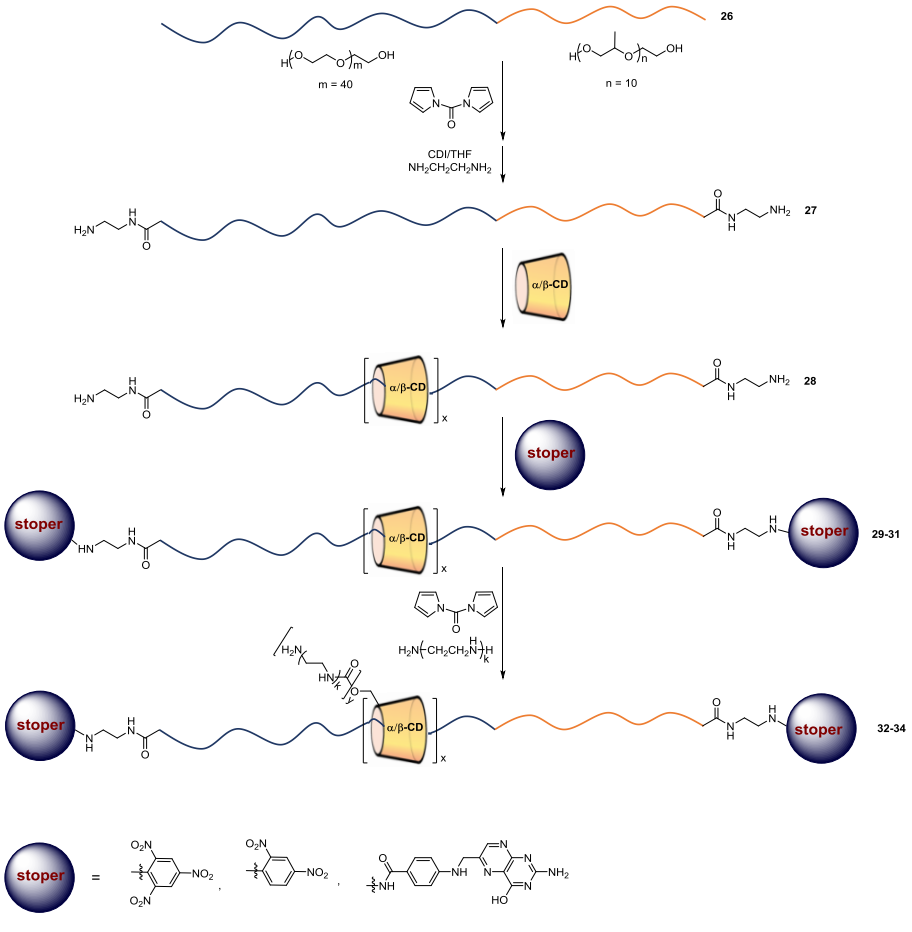


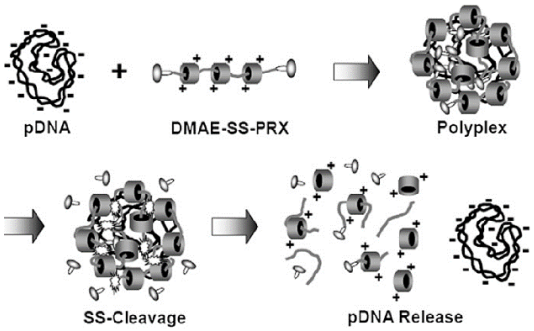
Fig. 17. García Fernández et al. prepared CD clusters

3.1.3 Cationic polyrotaxanes applied as DNA delivery vectors

Ionic CD-based on polyrotaxane (PR) system applied as nucleic acids' delivery also investigated by numerous scientists, Li team reported a PR system as gene carrier to deliver the genes. The PR system contained poly[(ethylene oxide)-(propylene oxide)] as axle, α-CD as cationic groups (pentaethylenehexamine salts) carry platform and two molecules of 2,4,6-Trinitrobenzene sulfonic acid (TNBS) as terminated stoppers (**32**). (See scheme 6) Subsequently, the prepared cationic PR system formed complexes with pDNA further to transfect cells[103]. Then they substituted the axles with PEG and the stoppers with 2,4-dinitrobenzene (**33**), formed similar cationic PR which possessed similar delivery properties as the above cationic PR system.[125] Next β-CD as cationic groups and larger stoppers were added as the lager ring cycle size of β-CD[104]. With a similar strategy, Chen team applied the folate as stoppers (**34**) and at the same time as target to folate receptors, over expressed on numerous cancer cells surface[105] which is a very brilliant strategy that could improve the delivery efficiency with the help of targeting effect. (See scheme 7) In order to control the release of the delivered pDNA, Harashima team introduced disulfide linkages at both terminal sides before the stoppers that could control the release of the pDNA via the S-S bond cleavage and improve the stability of PR gene delivery system[126]. The S-S bond could be ruptured under such as GSH rich environment which enriched in tumor microenvironment, which would provide a practicable tumor-targeted gene therapy delivery method.



Scheme 6. Synthesis of CD-based polyrotaxanes with different stoppers



Scheme 7. pDNA coassembly with dissulfide CD rotaxanes to form polyplex then controlled releasing pDNA via the S-S bond cleavage[106]

3.2 RNAs' delivery

In the human genome there over 95% of DNA sequences are non-protein-coding sequences[127], while many of them would translate massive functional non-coding RNAs (ncRNAs) which may regulate different functions physiologically. Interestingly, the ncRNAs themselves or mRNA may be also targeted by the RNA entities such as antisense RNAs (asRNAs), micro RNA (miRNAs), small interfering RNAs (siRNAs), and other small RNAs (sRNAs) that can manipulate the expression or function of target gene subsequently, control diseases[128–130]. This mechanism is generally called RNA interference (RNAi) holding vast therapeutics potential in cancer, autoimmune diseases, dominant genetic disorders, viral infections or any disease caused by the abnormal activity of genes[131]. The instances were reported by the Davis[115] team, first RNAi introduced gene silencing in human skin cancer in phase I clinical trial, demonstrated that the therapy successfully suppressed the expression of the cancer gene in 2010 and the first RNAi-related drug *patisiran* approved by the USFDA in 2018[132]. An appropriate delivery system is required intensely by the DNAs therapy which is also a vital factor to the RNAs therapy.

3.2.1 Targetable cationic polymers applied as siRNA delivery vectors

A classic instance for siRNA delivered by CD-based nanoparticles was reported by Davis et al. in 2007, they prepared CD-based polycation (similar as previously described in DNA delivery section), polyethylene glycolyzed adamantine (AD-PEG) and AD-PEG-transferrin (AD-PEG-Tf) as components of the nanoparticles[113,114,133]. PEG has been applied in many different types of non-viral gene delivery vectors, demonstrated that it's helpful for increasing the serum and salt stability. On the other hand, however, the PEGylation of nanoparticles reduced the interaction between the particles and cells, consequently, reduced the transfection of delivery vectors. On the consideration of this, the Davis team introduced the Tf, which is able to bind the Tf receptors (TfR) on the surface of cancer cells[134], on the vectors to hedge the transfection reduction brought by the PEGylation[116]. This was demonstrated by the delivery of the siRNA, an inhibitor of a subunit M2 of ribonucleotide reductase (RRM2), in the phase I trial of human skin cancer mentioned above[115]. (See fig. 18) The ribonucleotide reductase (RR) plays a key role in DNA replication and repair, hence the inhibition of RRM2 can provide the inhibition of RR that induces the apoptosis of the cancer cells[135].

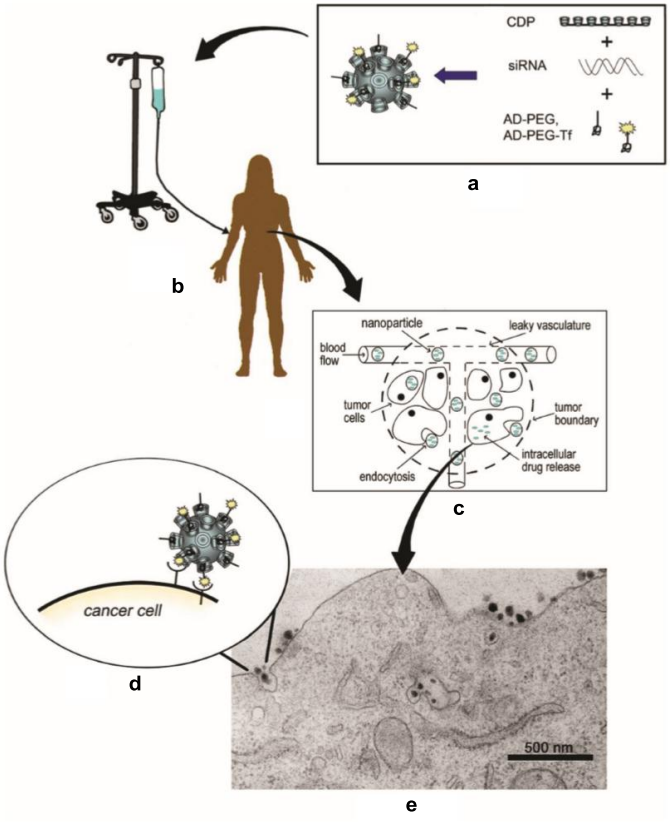


Fig. 18 a) siRNA contained assembled nanoparticles, then b) the aqueous solution of nanoparticles injected to trial subjects, c) via the EPR effect the nanoparticles were "leaking" from the micro blood vessels to the tumor tissues, d) and e) based on the Tf and Tf acceptor interaction, the nanoparticles were selectively binding to tumor cells.[116]

Similar strategy was taken by Wang[117] teams, that was applied cationic CD-based polymer to assemble nanoparticles with siRNA then via supramolecular chemistry to anchor folate (FA) that could specifically target on folate receptor (FR), consistently high and uniform expression on numerous cancer cells[136], to obtain the siRNA delivery vectors. On the primary face of β-CD were introduced disulfide contained cationic groups to achieve β-CD-based cationic polymer[118]. Notably, the disulfide structure could be used as a trigger for the endosomal/lysosomal escape of loaded siRNA in a reductive environment, or the siRNA would not be released. The folate was linked to adamantane via linear PEG to form FA-PEG-Ad, in which the adamantanes could interact with the previous prepared (PCD^{+})s to form host-guest complexes. (See fig. 19) The cytotoxicity, hemolysis, stability and siRNA transfection assays suggested satisfying results.[117]

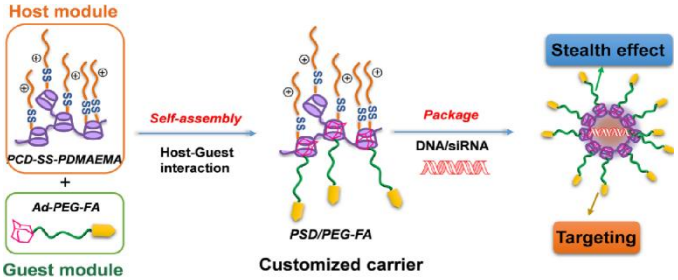


Fig. 19. 4-component of nanoparticles delivering siRNAs[117]

Acute myeloid leukemia (AML) is a deadly clonal disorder disease which with great potential being cured by the RNA interference (RNAi) therapy[137], however the nonspecific tissue distribution, poor cellular transfection, toxicity and short plasma half-life time et al. hedged the RNAi therapy in clinic[138]. Therefore, the O'Driscoll team developed an antibody targeted CD-based siRNA delivery system for the AML's treatment[119]. In this system, firstly, they applied the CD as platform grafted cation groups then with the siRNA formed CD/siRNA complex[139]; secondly, the 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000] (DSPE-PEG-Maleimide) conjugate was prepared[140]; tertiary, prepared the DSPE-PEG-Fab (a monoclonal antibody specific target to IL-3Rα, a cell surface antigen for human AML leukemia stem cells (LSCs))[141]; finally, CD/siRNA and DSPE-PEG-Fab were incubated at 60°C for 1 h with slight shaking, to give the CD/siRNA/DSPE-PEG-Fab formulation nanoparticles[142]. The mechanism of targeted nanoparticles delivered the siRNA via Fab bind to IL-3Rα elicited endocytosis, subsequently, endosomal/lysosomal escaped and released siRNA. Then, the siRNA activated RNAi effect to downregulate the corresponding gene (BRD4, an epigenetic reader[143]) induced the leukemia apoptosis. (See fig. 20) *In vitro*, the gene silencing in KG1 cells (an AML leukemia stem and progenitor cell line), the targeted nanoparticles exhibited efficiently and selectively delivery of siRNA to silence BRD4 induced leukemia apoptosis, in addition, the nanoparticles also could combine with clinically available chemotherapeutic Ara-C and reveal the combination therapy for AML treatment[119].

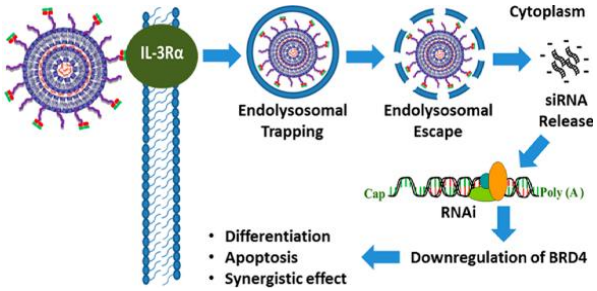


Fig. 20. Transfection mechanism of IL-3Rα targeted siRNA delivery systems based on CDs[119]

3.2.2 Cationic polyrotaxanes applied as siRNAs delivery carriers

Thompson et al.[144] devised α-CD-based cationic polyrotaxanes for the delivering the siRNA. They prepared three polyrotaxanes derived from three different sizes of polymer axles (PEG MW 2,000, 3,400, and 10,000). The cationic polyrotaxanes were synthesized via two steps: 1st, different sizes of axles were mixed with the free α-CD to form polyrotaxanes (CD-PR), then end-capped with 2,4,6-Trinitrobenzenesulfonic acid (TNBS); 2nd, the CD-PR reacted with N,N'-dimethylethylenediamine (DMEDA) to obtain the cationic polyrotaxanes (PR^+)[103,104,107,125]. (See fig. 21) These PR^+s were able to condense siRNA into positively charged particles with a diameter smaller than 200 nm. The cell viability assay and cellular uptake studies of PR^+ nanoparticles compared to bPEI displayed 100 to 200 times less toxic and even better cellular uptake percentage during the N/P ratio was 20[144].

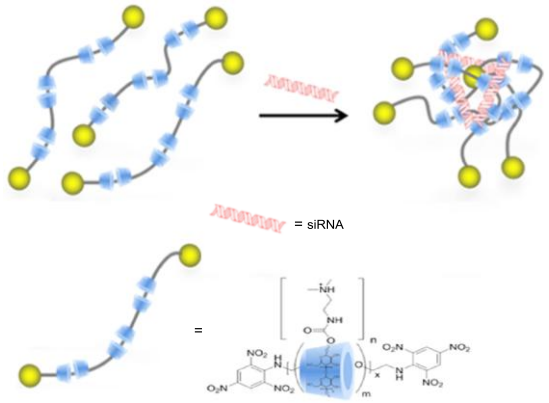


Fig. 21. Polyrotaxanes co-assembly with siRNAs form complexes for delivering of siRNAs[144]

3.2.3 Cationic amphiphilic monomers applied as siRNAs delivery carriers

In order to overcome the issues in delivering siRNA into neurons and the central nervous system, like neuronal uptake, vesicular escape, and blood-brain barrier et al.[145,146] to treat some nervous system diseases, O'Driscoll and Cryan teams collaborated to devise a neuronal siRNA delivery carrier based on modified β-CD[139]. They introduced lipophilic groups on the primary face and the cationic groups via "click chemistry" on the secondary face of β-CD selectively to form a functionalized CD monomer[111]. (See fig. 22) Then the candidate siRNAs were formed nanoparticles with the CD monomers, sized 200 nm approximately. Several experiments were launched to evaluate the delivery efficiency, such as serum stability, cell viability, cellular uptake and transfection efficiency via the related gene knockdown technique *in vitro*. The results revealed that, in serum condition the cargos were stable, the cells maintained 80% of viability after the transfection trials and significant gene knockdown was observed via the studies of reduction of luciferase and glyceraldehyde phosphate dehydrogenase (GAPDH) expression of up to 68% and 40%, respectively.[139]

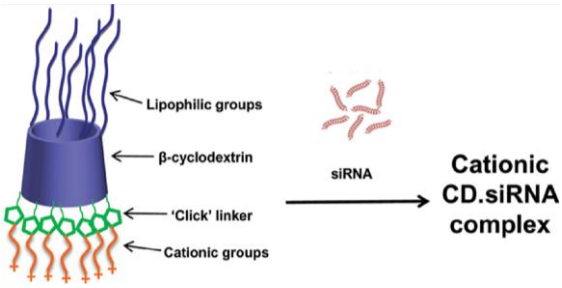


Fig. 22. siRNA formed complexes with CD-based amphiphilic cationic monomers[111]

3.2.4 A virus fabrication manner simulating RNA loaded method applied as siRNAs delivery carriers

An interesting and intelligent RNA delivery method probably is simulating the manner of virus in RNA transfection. Inspired by tobacco mosaic virus (TMV) were assembled from nucleic acids (NAs) and coat protein. Namely, the NAs were cooperative assembly with the coat protein, then to form NAs inside fiber shape virus, Sollogoub[120] team carefully mimicked the TMV assembly manner, designed a functional β-CD co-assembled with siRNA to form a fiber shape siRNA vector. They built a 4 carbon chain bridge on the primary rim of β-CD on $6^{A,D}$ positions via benzylation and selective bisdebenzylation[147,148]. Then an adamantane was grafted on 6^{A} position of β-CD

via reductive amination with 1-adamantaneacrtalhydride which could allow adamantane-β-CD (Ad-CD) co-assembled with sRNA to form the siRNA contained vectors. Notably, the introduced bridge could prevent the adamantane grafted sugar unit tumbled that led the self-inclusion and the head to head dimerization effect (see fig. 23). The gene silencing trials exhibited the vectors had satisfied siRNA transfection[120]. Compared to the above examples this strategy was more tunable and controllable in assembly to form better orderly delivery vector shapes (not just some nanocomplexes).

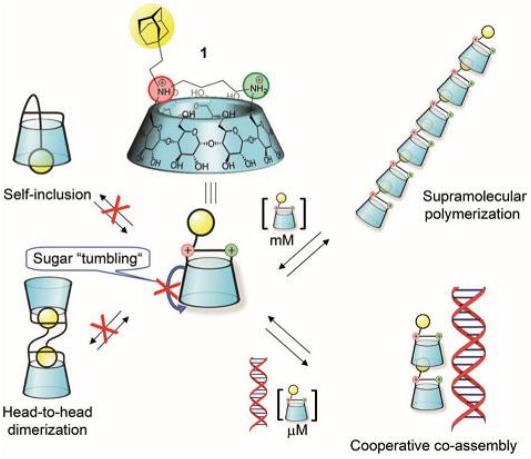


Fig. 23. siRNA co-assembly with supramolecular polymers[120]

4. Conclusions

Both proteins and NAs drugs play vital roles in the diseases' therapy now but more vital especially in the future that we can imagine. However, one of the most robust barriers that could slow down the future come to us earlier probably is the deficiency of efficient delivery systems. As for this big issue, CDs based delivery carriers can be strong candidates that become very potential solutions.

In theoretically, the proteins with hydrophobic groups can form host-guest interaction which is able to improve the ability of nanocomplexes formation between the proteins with CDs and the stability that largely preserve the activity of proteins and optimize the proteins' transfection that has been proved via the above examples. However, in terms of our knowledge, cyclodextrin based delivery systems are not very common delivery devices for proteins such as BSA[44], IgG[51], insulin[27], etc., especially, considered the giant numbers of proteins discovered. A proposed mechanism for explaining this phenomenon is probably that the complicate stereo-structures that impeded the CD-protein complex formation even with the existence of the host-guest interaction effect.

Compared to proteins, the NAs delivery based on CDs are much more popular and successful, especially, after the success of Davis[115] team promoted the siRNA for the skin cancer treatment to phase I clinical trial. In the early stage, the CDs applied as a platform to graft numerous of cationic groups to interact with the anions of NAs to form nanocomplexes for NAs delivery. Recently as the raising of CDs applied in supramolecular chemistry and more precise selective functionalization methods developed, the scientists started to assembly nano structures to mimic the "coat proteins" of the virus to carry the NAs, then transport the NAs to the desire spots[120].

While numerous disadvantages would be overcome, as the continuing progresses achieved in the

functionalization techniques in CDs[149], easier tunable and more appropriate CD-based materials as delivery devices for individual protein delivery will become more efficient and common.

As the burst of COVID19 pandemic, before the wonder drugs and vaccines (gen and protein vaccines) approved, the monoclonal antibodies are the most effective cures, hence, CDs can be functionalized to appropriate delivery systems for antibodies or gen and protein vaccines[150].

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