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FEATURE ARTICLE

Supramolecular cyclodextrin-based drug nanocarriers

Susana M.N. Simões[a,b], Ana Rey-Rico[c,d], Angel Concheiro[c], Carmen Alvarez-Lorenzo[c,*]

5 Supramolecular systems formed by the binding of several cyclodextrins (CDs) to polymers or lipids, either via non-covalent or covalent links, open a wide range of possibilities for the delivery of active substances. CDs can perform as multifunctionalizable cores to which very diverse (macro)molecules and drugs can be conjugated. Grafting with amphiphilic molecules can lead to nanoassemblies exhibiting a variety of architectures. CDs can also polymerize with other CDs or can be used to functionalize 10 preexisting polymers to form polymers/networks with enhanced capability to form inclusion complexes. Alternatively, CDs can be exploited as transient cross-linkers to form poly(pseudo)rotaxane-based networks or zipper-like assemblies. Combination of mutifunctionality and complexation ability of CDs has been shown useful to develop depot-like formulations and colloidal nanocarriers with improved performances regarding easiness of administration, protection of the encapsulated substances, control of 15 the delivery rate, and cell interactions. The aim of this review is to provide an overall view of the diversity of designs of CD-based supramolecular nanosystems with a special focus on the advances materialized in the last five years, including clinical trials.

1. Introduction

Pharmacological activity of a drug is the result of affinity and 20 interaction with the biological target, but also of optimum exposure at the target site. It is therefore necessary for the drug to reach the site of action following administration (i.e., oral, intravenous, transdermal, etc.) at sufficient concentration, avoiding nonspecific uptake and rapid clearance from the blood 25 stream. Supramolecular cyclodextrin (CD)-based drug carriers may be a versatile approach for materializing these aims. Among the many strategies explored to enhance drug solubility and stability and regulate release rate, and thus bioavailability,[1,2] formation of inclusion complexes with CDs has been extensively 30 applied.[3-8] In fact, the use of CDs as individualized entities already have a long history in pharmacy.[9] More recently, the design of novel systems in which the CDs act cooperatively to further exploit the host-guest interactions, mimicking the relatively weak but redundant interactions of molecular 35 recognition in Nature, is opening an unexpectedly wide range of advanced applications.[10-13]

CDs are a group of cyclic oligosaccharides obtained from enzymatic processing of starch with a torus-like molecular shape. These cyclic oligosaccharides containing six (αCD), seven 40 (βCD), eight (γCD), or more (α-1,4-)-linked D-glucopyranose units, consist of a relatively hydrophobic inner cavity and a hydrophilic outer face. Functionalization of the external hydroxyl groups of natural CDs with short alkyl chains minimizes self-aggregation through hydrogen bonding interaction,[14] and enables 45 preparing derivatives of natural α-, β- and γ-CDs with remarkably greater aqueous solubility. Together with natural CDs,

hydroxypropylated-βCD (HP-βCD), hydroxypropylated-γCD (HP-γCD), randomly methylated-βCD (RM-βCD) and sulfobutyl ether βCD sodium salt (SBE-βCD) are regarded as safe (GRAS) 50 and included in monographies at various national pharmacopeias[15], although threshold doses above which adverse effects may appear depending on the administration route are being reevaluated.[16]

One of the most interesting attributes of CDs is their ability to 55 host a variety of lipophilic compounds which can be partially or totally included in the hydrophobic internal cavity; the hydrophilic outer face being exposed to the aqueous medium.[6,17] The phenomenon of CD inclusion complex formation is a multifaceted process driven by many factors, mainly due to 60 displacement of enthalpy-rich water molecules from the CD cavity (repulsive polar-non polar interactions) by a "guest molecule" with appropriate geometry and physicochemical properties.[18] The predominant interactions engaged in the drug-CD complex include van der Waals and hydrophobic binding, but 65 other such as hydrogen bonding, release of ring strain in CD and change in solvent-surface tensions may be also involved.[19,20] Nevertheless, CDs are not restricted to host-guest interactions, and other phenomena like non-inclusion complexes or nanostructures formation do occur in aqueous CD solutions.[21-24] 70 Hosting the drug in the inclusion and non-inclusion complexes increases its apparent solubility and facilitates formulation of solutions and also of immediate release solid dosage forms.[25] Moreover, CDs may also improve drug bioavailability by affecting permeability through biological membranes,[18,26] and by 75 controlling the rate and/or time profile of drug release.[27,28] The increase in the dissolution/permeability performance can result in the improvement of oral bioavailability of class II and IV drugs

of Biopharmaceutical Classification System (BCS), enhancing the pharmacological effect and allowing a reduction in drug dose.[8,17,29,30] CDs have been also used to minimize gastrointestinal and ocular irritation, to mask smells or tastes, to
5 avoid interactions among different drugs and/or specific excipients, to lower compound volatility, and to transform oily substances into microcrystalline or amorphous powders.[17,31] Incorporation of CDs to nanostructured networks may result in carriers that exhibit distinct hydrophobic/hydrophilic domains
10 useful for hosting water-soluble or insoluble drugs.[32-34]

CDs have been found to form inclusion complexes not only with low molecular weight drugs, but also with macromolecules. New molecular structures and functions have been obtained combining polymers and lipids with CDs (Fig. 1). These
15 supramolecular assemblies are valuable models for understanding the mechanisms of molecular recognition,[35-37] and also provide structures useful for designing novel biomaterials.[38-42] Harada and co-workers[43] first reported in the 90´s on an inclusion complex formed via threading of αCD along poly(ethylene glycol) (PEG)
20 chains. Since this pioneering finding of CD-polymer inclusion complexes, several other polymers bearing separate hydrophilic and hydrophobic moieties or blocks have been observed to form inclusion complexes with various CDs.[44-46] Versatile design and tunable features of poly(pseudo)rotaxanes explain the great
25 attention that these supramolecular structures are receiving in the biomedical field.[47-50] In parallel, the synthesis of CD-polymers (poly-CDs) has been developed to a large extent with the purpose of combining the advantages of the polymers (high molecular weight and targeting capability) with the ability of CD to form
30 inclusion complexes[51] (Fig.1). Most of the publications deal with the synthesis of βCD-polymers since βCD interacts with the widest range of drugs,[11,52-55] but recent research has focused on γCD- and αCD-polymers due the appropriated cavity size of γCD to host large molecules,[56-58] and the ability of poly-αCD to tread
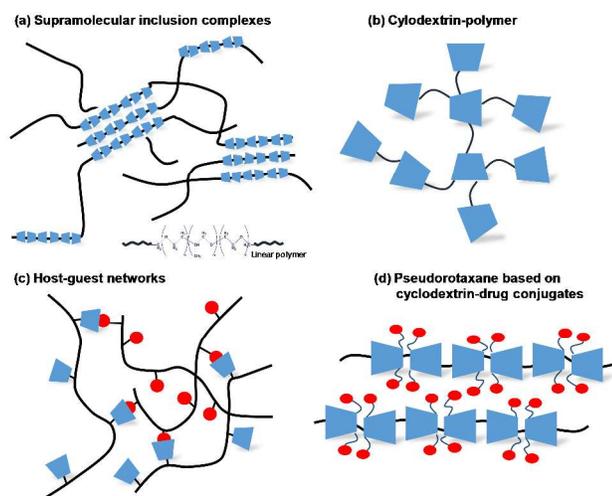35 along PEO chain forming syringeable gels.[59]

Fig. 1 (a) Schematic structure of supramolecular CD-based inclusion complexes, (b) representative structure of a CD-polymer, (c). supramolecular host-guest networks and (d)
40 pseudorotaxanes based on CD-drug conjugates.

Interestingly, polymers with grafted CDs can also lead to supramolecular architectures through host-guest interactions with polymers functionalized with suitable guests, such as adamantane
45 or imidazole, resulting in zipper-like assemblies.[60-62] More recently, supramolecular nanomedicines in which the drug is covalently linked to functionalized CDs have revealed particularly suitable for addressing a variety of delivery demands.[63]

50 The aim of this review is to provide an overall view of the diversity of designs of supramolecular nanosystems that have been prepared using CDs as free entities, grafted to specific (macro)molecules or covalently linked each other, for the delivery of active substances. There is already a large number of
55 publications in this field, and some comprehensive reviews have analyzed the main findings mostly up to 2010 or so.[24,36,39,64] Thus, the present review aims to cover the achievements of the last five years, although in the context of the previous knowledge. Since the number of papers is exponentially increasing, we have tried to
60 gather relevant information according to the main interactions involved in the formation of the supramolecular structure: i) CDs threading along polymers and subsequent polypseudorotaxane assembly, ii) host-guest assemblies between macromolecules bearing complementary ligands, iii) self-assembly of native or
65 amphiphilic CDs with amphiphilic lipids or polymers, and iv) covalent conjugates of drugs with functionalized CDs. Formulations in clinical trials are devoted a particular attention.

2. Cyclodextrin-based poly(pseudo)rotaxanes

70 CDs can thread along certain polymer regions (main-chain complexes) or lateral chains (side-chain complexes) leading to supramolecular assembled structures. Polyrotaxane term refers to supramolecular systems in which the CDs are trapped on certain regions of the polymer and cannot freely move along the chains;
75 this is the case of polymers containing bulky regions (stoppers) that are synthesized in the presence of CD units. Oppositely, polypseudorotaxanes lack of stoppers and CDs can reversibly travel along the polymer backbone or lateral chains. General concepts and applications of polyrotaxanes and
80 polypseudorotaxanes have been addressed elsewhere.[64-67] The enthalpy-favored threading process of CDs along polymer chains is driven by van der Waals and hydrophobic interactions, as well as hydrogen bonding among neighboring threaded CD units.[68] The interaction of CD-based poly(pseudo)rotaxanes with water
85 molecules notably impact on their mechanical properties and reactivity, which in turn determines their applications in the drug delivery field.[41,67,69] Moreover, CD-based supramolecular systems can be endowed with further performances such as targeting to specific tissues or cells by decoration with
90 carbohydrate ligands, peptides, or antibodies.[70]

CD-based polypseudorotaxanes generally consist of polymers such as poly(ethylene oxide) (PEO) or PEG,[71,72] poly(propylene oxide) (PPO),[73,74] or copolymers having blocks of PEO and/or PPO, solely like PEO-PPO-PEO (Pluronic®)[75-77] or combined
95 with other blocks such as poly[(R)-3-hydroxybutyrate][78] or poly(caprolactone) (PCL).[79] αCD units can thread along PEO blocks with a stoichiometry of 2 EO units per one αCD; polypseudorotaxane formation is even possible using reverse

Pluronics (i.e., with the hydrophobic blocks at both ends, PPO-PEO-PPO) as αCD can slid over PPO blocks to selective accommodate along the EO units.[36,80] βCDs do not form complexes with PEO but with PPO[43] (Fig. 2). In the case of γCD,
5 two chains of PEO can be simultaneously threaded through each γCD cavity,[74,81] and the complexes can be even formed although PEO is not the end block of a copolymer.[79] PPO also forms inclusion complexes with γCD.[73,82] Other polymers, such as polyethyleneimine (PEI)[83] and polyphosphoester ionomers[84] are
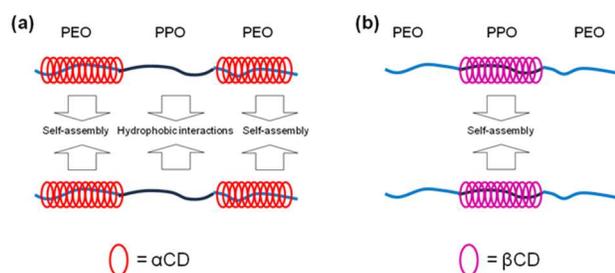10 also suitable candidates to be threaded by CDs.

Fig. 2 Structure of polypseudorotaxanes of PPO-PEO-PPO triblock copolymers (Pluronic) and αCD (a) and βCD (b).
15 Reprinted with permission from Larrañeta and Isasi[75]. Copyright 2012 American Chemical Society.

Intermolecular interactions among CDs threaded along different polymer chains may result in the formation of
20 superstructures that notably modify the rheological properties of the system.[84,85] When a concentrated aqueous solution of αCDs is mixed with PEG or PEO-block copolymers, the mixture rapidly becomes turbid. The cooperative threading of a number of αCD molecules onto the hydrophilic polymeric chain causes the
25 dehydration of the chains.[86] Then, threaded αCDs stack forming nanocylinders with a crystalline channel type structure in which PEG is included.[43] This process is entropically unfavorable, but enthalpically driven due to hydrogen bond formation between αCDs that arrange head-to-head and tail-to-tail along the
30 nanocylinder. Polypseudorotaxane formation becomes faster as PEG molecular weight increases up to 1000 Da, but beyond that molecular weight the rate reaches a plateau and then progressively decreases. Association of the threaded CDs from adjacent polypseudorotaxanes may lead to phase separation or to
35 a three-dimensional network, depending on the polymer and its concentration as well as on the temperature and ions content of the medium.[77,87,88] Interestingly the inter-polypseudorotaxane interactions are minimized when hydrophilic derivatives of the natural CDs are used; namely, hydroxypropyl-CDs can thread
40 onto the polymers but do not stack. Thus, they form hydrosoluble polypseudorotaxanes that do not significantly modify the turbidity/viscosity of the system.[75,89]

Association of CD nanocylinders into microcrystals acts as tie-junction for gel formation.[76] Relatively high concentrations of
45 both the CD and the polymer and/or the use of high molecular weight polymers facilitate the formation of precipitated domains, which serve as cross-linking points. Since the inter-

polypseudorotaxane interactions are reversible, the supramolecular gels exhibit thixotropic behaviour and, when
50 subjected to shear stress, the viscosity greatly diminishes.[45] Thus, the system undergoes gel-to-sol transitions by simple application of an external pressure, and behaves as a formulation injectable through a common needle. At rest, the viscosity of the gel is recovered and a depot is formed in the injection site.[36] Compared
55 to the αCD-PEG systems that require several hours in being formed and in restoring the initial viscosity after shear stress, polypseudorotaxanes obtained combining αCD and amphiphilic block copolymers such as PEO-PPO-PEO[77] or PCL-PEG-PCL[90] rapidly lead to gels that form and reform faster, probably because
60 of the additional contribution of the interactions among hydrophobic blocks. For example, compared to Pluronic F127 (PEO_{100}-PPO_{69}-PEO_{100}) solely systems that require a concentration close to 20% to form temperature-responsive gels, addition of αCD (5%) leads to gels with copolymer concentration
65 as low as 6.5%.[77] Increasing the concentration of both components, the gel can be obtained in few minutes. These polypseudorotaxane-based gels sustained vancomycin release for several days being active against to *Staphylococcus aureus* in *in vitro* cultures.[77] On the other hand, combination of αCD (12%)
70 and PCL-PEG-PCL (10%) causes gel formation in less than one minute and enables sustained release of vitamin B_{12} for at least 20 days.[90] Similarly, strong gels have been obtained mixing αCD with nucleobase (adenine/thymine)-terminated PEG, because hydrogen bonding between adenine and thymine act as additional
75 crosslinking points. Studies carried out with U14 cancer cell xenograft-bearing mice evidenced that doxorubicine-loaded gels inhibit tumor growth more efficiently than doxorubicine solutions and αCD/PEG gels.[91]

The mild conditions under which polypseudorotaxane-based
80 gels are prepared allows the incorporation of a variety of active substances, also comprising hydrophobic molecules that can benefit from the presence of free CDs or micelle-like structures available for drug hosting.[41,87,92,93] For example, mixing PEG-PCL micelles that solubilize doxorubicin, PEG-PAA
85 (poly(ethyleneglycol)-*b*-poly(acrylic acid)) micelles that host cisplatin, and αCD all together, a dual-drug loaded hydrogel is obtained. The gel properties have been shown to depend on the length of PEG blocks and the additional incorporation of PEG homopolymer or Pluronic copolymers. The erosion of the gels
90 releases discrete micelles from which the drugs are delivered.[94] A similar strategy was followed to prepare gels for the sustained release of camptothecin (CPT) and granulocyte colony-stimulating factor (G-CSF) from heparin-conjugated Pluronic® micelles.[95] Supramolecular gels of CPT have been recently
95 prepared from aqueous dispersions of CPT conjugated to low molecular weight PEG to which αCD was added. Partial inclusion of PEG chains and hydrophobic aggregation of CPT ends resulted into stable gels, which could also incorporate other hydrophilic antitumor agents (such as 5-fluorouracil).[96] These
100 gels showed temperature-tunable controlled release of both drugs (Fig. 3).
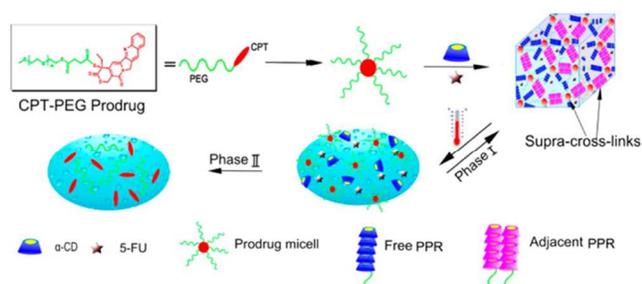
Fig. 3 Camptothecin (CPT)-PEG conjugates form stable hydrogels via hydrophobic aggregation of CPT groups and polypseudorotaxane formation of PEG and α-CD. 5-fluorouracil (5-FU), which is commonly combined with CPT for tumor treatment, can be loaded in the aqueous phase of the hydrogels. On-demand gel-sol transitions can be obtained as a function of temperature, regulating PEG chain length and αCD concentration. Reprinted with permission from Ha et al.[96] Copyright 2014 American Chemical Society.

Sustained release of proteins and gene material is also receiving an increasing attention.[97,98] Insulin has been shown to remain stable when incorporated into CD/PEG hydrogels (Abu Hashim et al., 2010). Addition of αCD (145 mg/mL) or γCD (232 mg/mL) to solutions containing PEG (100 mg/mL) and insulin (5.74 mg/mL) led to gels that showed prolonged release of the hormone. Polypseudorotaxanes of γCD formed more slowly, but provided more sustained release (Fig. 4).[99] Studies in animal models revealed that the insulin/γCD/PEG gel significantly decreased glucose serum levels and prolonged the hypoglycemic effect for 12 h, compared to the insulin/αCD/PEG hydrogel that caused the minimal glucose levels at about 2 h after injection and then basal level was recovered within 6 h. Alternative gels for insulin controlled release involved the use of γCD (10.54%) and PCL-PEG-PCL (2.5%).[79] Gelation occurred within one minute, and the gels showed an excellent syringeability and sustained insulin release in vitro for more than 20 days.

Gels of αCD with methoxyPEG-poly(ε-caprolactone)-(dodecanedioic acid)-poly(ε-caprolactone)-methoxyPEG triblock polymer (αCD/MPEG-PCL-MPEG) have been shown biocompatible and able to slowly release recombinant human erythropoietin (rhEPO) in an acute myocardial infarction rat model.[100] The rapid gelation of this system enabled effective encapsulation of rhEPO at the injection site, which improved cardiac function for 30 days after myocardial infarction, and avoided polycythaemia, a well-known collateral effect of rhEPO.[100,101] On the other hand, methoxyPEG-poly(ε-caprolactone)-poly[2-(dimethylamino)ethyl methacrylate] triblock polymer (MPEG-PCL-PDMAEMA) form stable polyplexes with plasmid DNA. Adding αCD to the polyplexes system leads to injectable polypseudorotaxane gels, which control plasmid release for several days without detrimental effects on protein expression levels.[92] Interestingly, responsive gels have been prepared combining αCD and a copolymer formed by blocks of monomethyl ether PEG and poly(amido amine) connected through a disulfide bond (mPEG-g-SS-PAMAM). In the absence of a reducing agent (normal tissue) the gel provided drug sustained release, but at high concentration of reducing

agents (mimicking pathological tissue or intracellular milieu) the supramolecular gel disintegrated and the release rate accelerated.[102]
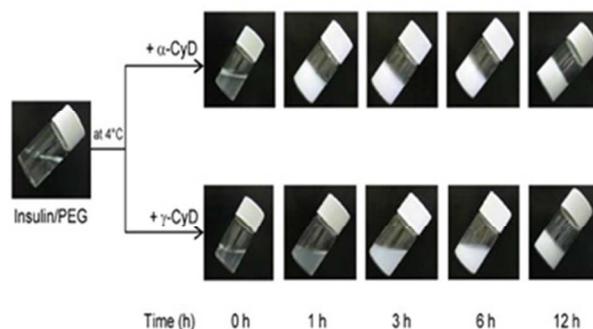

Fig. 4 Effect of CD type on incubation time required for formation of insulin/CD/PEG hydrogel systems. Adapted from Abu Hashim et al.[99] with permission from Elsevier.

Most publications on polypseudorotaxanes refer to the formation of inclusion complexes of CDs with linear polymers, but they can be also formed with X-shape block copolymers like poloxamine (Tetronic®). Their unique architecture in four arms of PEO-PPO connected to a central ethylenediamine group determines that the self-assembly as micelles or gel structures depends not only on polymer concentration but also on the pH and temperature of the medium.[103,104] One poloxamine variety, Tetronic 908 (T908, with 114 EO and 21 PO units per arm), has been shown able to first induce in vitro proliferation of mesenchymal stem cells and then trigger differentiation to osteoblasts.[105] Syringeable gels with osteogenic capability have been prepared combining αCD (5-7% w/v) and T908 (as low as 2%) also incorporating simvastatin hydroxy acid as osteogenic coadjuvant.[106] The polypseudorotaxane-based gels were shown able to increase drug solubility and sustain the release for more than one week, particularly those formed with high αCD concentration (9.7%). These syringeable synthetic scaffolds showed outstanding osteoinductive effects (Fig. 5).[106]
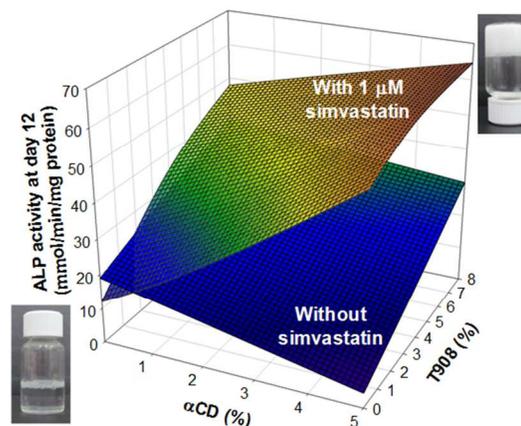

Fig. 5 Dependence of alkaline phosphatase activity (ALP) of mesenchymal stem cell cultures on the composition of polypseudorotaxane-based gels combining αCD, poloxamine

T908 and simvastatin. Reprinted from Simões et al.[106] with permission from Elsevier.

A variety of nanocarriers can be created exploiting the self-aggregation of CDs forming part of polyrotaxanes. For example, polyrotaxanes of cinnamic-acid-modified PEG chains self-assembled as supramolecular polymersomes that transformed into micelles once doxorubicin was loaded. The supramolecular nanostructures exhibited better tumor growth suppression in an *in vivo* tumor model than the free drug.[107] Nanoparticles with a core of αCD polyrotaxanes and a shell of low-fouling PEG chains have been obtained from polyrotaxanes in which PEG chains were capped with bulky groups through disulfide bonds and subsequently modified via click chemistry for the grafting of additional PEG chains (Fig. 6). The threaded αCDs assembled forming a compact core suitable for encapsulation of hydrophobic drugs. The obtained core-shell nanocarriers exhibited the unique feature of being responsive to intracellular glutathione levels, releasing the payload inside cells.[108]
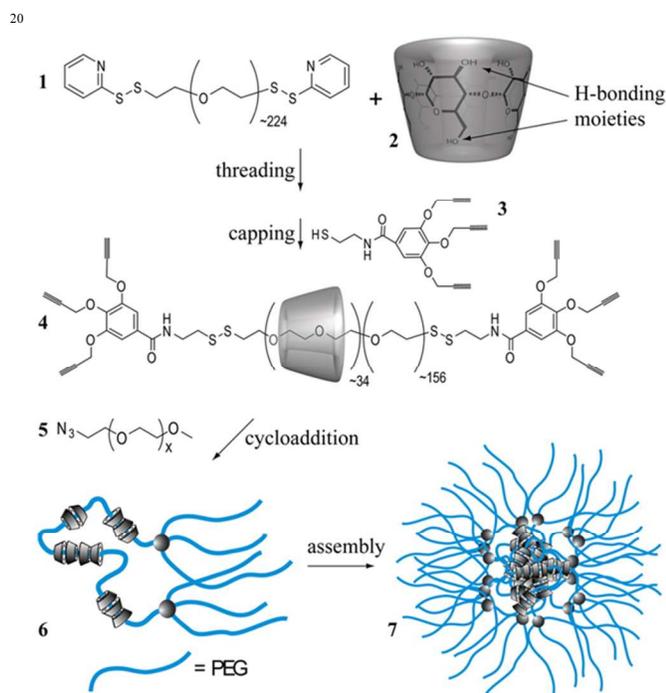

Fig. 6 Schematic draw of the end-capping and further grafting of αCD-PEG polyrotaxanes with PEG chains using alkyne-azide click chemistry. The triblock polyrotaxanes self-assembled in water into core-shell particles via intra and intermolecular H-bonding between threaded αCDs. Reprinted with permission from Tardy et al.[108] Copyright 2014 American Chemical Society.

3. Cyclodextrin polymers

CD-polymers have been explored for the last 30 years due to their great versatility, since the molecular weight, architecture (linear vs. branched) and pendant ligands can be readily tuned.[109] CD-polymers preserve the capability of CDs to form inclusion complexes, which may be even enhanced due to cooperative effects.[110,111] Linear and branched CD polymers can be prepared

in different ways:[41] (1) condensation of CDs with bi- or multifunctional cross-linkers, such as epichlorohydrin (EPI), biepoxides, diisocyanates or polyacids;[112-114] (2) anchoring of CDs to polymer chains;[115] and (3) polymerization of acrylic/vinyl derivatives of CDs with other monomers.[116] Details of reaction conditions and yields have been reported elsewhere.[22,117-119]

The first approaches to pharmaceutical application of CD-polymers (either water soluble or insoluble) referred to copolymers of CD and EPI,[19,120] which lead to quite soluble inclusion complexes. EPI/βCD molar ratio, NaOH concentration, and reaction time are main variables affecting polyCDs molecular weight.[113] Low NaOH concentrations promote substitution on the three possible positions of the CD cavity (OH-2, OH-3 and OH-6), while more concentrate NaOH medium favors substitution on one side of the cavity (OH-6). Some examples of the performance of poly-CDs on the solubility and bioavailability of hydrophobic drugs are collected in Table 1.[121-125]

TABLE 1 INSERT NEAR HERE

Hydrophilic EPI-crosslinked βCD polymers have been shown to increase apparent solubility of naproxen 30-fold compared to the drug aqueous solubility.[126] EPI-βCD polymers have been also able to accelerate dissolution and enhance oral bioavailability of glipizide.[55] The glipizide/poly-βCD complex exhibited 36.7 and 10-12 times higher solubility than pristine drug or its physical mixture with HP-βCD, respectively. Similar enhancements in drug solubility have been reported for hyperacin[127] and triclosan[128]. Triclosan exhibits low biological activity because its poor aqueous solubility, and several attempts to increase the solubilizing efficiency of βCD by incorporation of fixed amounts (1%, w/v) of hydroxypropylcellulose, hydroxypropyl methylcellulose or amidated pectin were carried out. Addition of these hydrophilic polymers to triclosan/βCD systems increased overall drug solubility, but decreased βCD complex ability towards the drug.[129] Interestingly, EPI-βCD polymers (Ks = 11,733 M^{-1}) showed higher solubilizing and complexing ability compared to native βCD (Ks = 2,526 M^{-1}). Such a greater stability constant may be attributed to the cooperative effects of adjacent CD cavities on drug interaction. Many other examples confirm that EPI-βCD copolymers can aid with solubilisation of a wide range of lipophilic molecules.[129,130]

Although still few, some studies have reported on the performance of poly-γCD and poly-αCD as carriers of hydrophobic drugs. Water soluble EPI-γCD polymers with molecular weights ranging from 10^4 to 10^6 g•mol^{-1} exhibit interesting complex forming properties, as evidenced using methyl orange and sodium fusidate as guests (association constants between $6•10^3$ and $4•10^4$ M^{-1}).[57] On the other hand, water soluble EPI-αCD polymers (Mw 4.55•10^5 g•mol^{-1}) can form supramolecular gels with PEG and PEO-PPO copolymers, e.g. Pluronic F127 and Tetronic 908. The PEO chains are threaded by the αCD units of poly-αCD leading to 3D-supramolecular gels that are triply cross-linked by the stacking of αCD units, the polymeric links among the CDs, and the hydrophobic interactions among PPO blocks.[59] These cytocompatible supramolecular gels showed promising features to develop syringeable systems able to sustain drug release under

physiological conditions.

Branched cationic βCDs polymers (cationic-polyβCDs) with designed chemical structures have been synthesized from βCD, EPI and choline chloride.[131,132] Drug loading occurred via inclusion complexes and ionic interactions, which were particularly intense for anionic drugs like indomethacin. In vitro release studies from calcium alginate hydrogels containing indomethacin-cationic-polyβCD complexes indicated that cationic-polyβCDs modulate indomethacin release through interactions with the drug molecules and the network.[132] Cationic CD-polymers have been also investigated as effective nonviral vectors for gene delivery.[36,132,133] Star polymers have been prepared via grafting of multiple oligoethylenimine (OEI) arms onto an αCD core.[133] Complexes of αCD-OEI polymers with plasmid DNA (pDNA) led to nanoparticles (100-200 nm) that were more cytocompatible and exhibited higher gene transfection efficiency than those observed for branched PEI (25K). Related γCD-OEI polymers with folic acid conjugated via a disulfide linker have been shown suitable for target co-delivery of paclitaxel (PTX) (as γCD guest molecule) and pDNA (as polyplexe) into cancer cells that over-express folate receptors.[134] Importantly, the redox-sensitive disulfide linker allows detachment of folic acid groups from the carrier after endocytosis, which facilitates the recycling of the folate receptors and thus a continuous folate-mediated endocytosis to achieve enhanced gene transfection (Fig. 7).
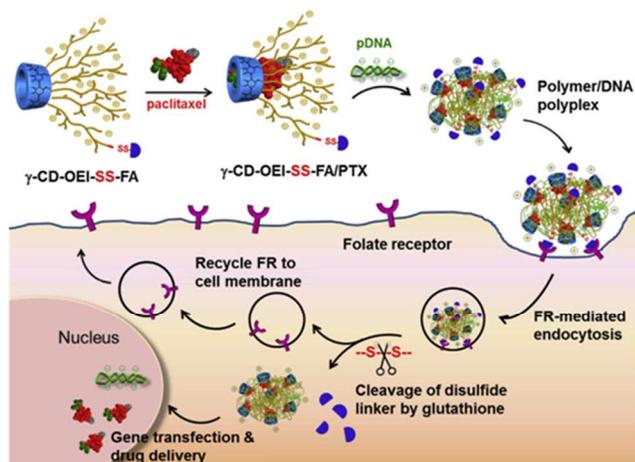

Fig. 7 Structure of γ-CD functionalized with OEI chains that bear folic acid linked through disulfide bonds, and interaction with paclitaxel (PTX) by means of inclusion complex formation and with plasmid DNA forming polyplexes with OEI. After endocytosis, drug and gene co-delivery occurs and folic acid is detached from the supramolecular self-assembly for an easier recycling of folate receptors. Reprinted from Zhao et al.[134] with permission from Elsevier.

CDs can be grafted to preformed polymers. For example, grafting CD molecules onto chitosan backbone can lead to mucoadhesive delivery systems[135] or scaffolds for tissue engineering,[136] that exhibit inclusion complex formation, bioavailability improvement, and specific mucosal targeting.

Biodegradable chitosan-g-βCD scaffolds prepared applying a freeze-drying method formed a matrix suitable for loading and controlled release of ketoprofen.[135,136] Morphology, swelling and drug release properties of the scaffolds depended on the cross-linking density. It has been recently observed that chitosan-g-βCD binds insulin through electrostatic and host-guest interactions forming supramolecular aggregates over a wide range of pH (3.6-7.4), which protects insulin against digestive enzymes. Depending on whether acetate, citrate or phosphate buffer is used, continuous networks, nanoparticles or large aggregates are respectively obtained.[137] In either case, the supramolecular structures can be reversible broken under mild forces, such as those occurring along gastrointestinal tract, which may open novel ways of addressing oral administration of protein drugs. In other study, βCD-conjugate to PEG-poly(L-glutamic acid) diblock copolymer has been shown to form spherical supramolecular complexes with camptothecin, enabling protection of the active lactone ring of the drug.[138]

4. Host-guest assembled systems

Mixing poly-CDs or polymers functionalized with grafted CDs and macromolecules bearing groups that can act as guests of the CDs may lead to supramolecular structures driven by zipper-like assemblies. Both gels and nanocarriers can be obtained depending on the architecture and total and relative concentration of the components.[116,139] Moreover, if the polymer chains have stimuli-responsive moieties, the properties of the zipper assemblies depend on the balance between attractive (inclusion complexes) and repulsive (triggered by the stimuli) interactions.[115,140,141] In either case, the zipper assemblies are reversible, and deformable/syringeable structures can be obtained.

Most zipper-based structures rely on βCD-bearing polymers and adamantine-bearing macromolecules, due to the very high host-guest association constant (Ka ~ 10^4–10^5 M^{-1}).[142] As an example, supramolecular hydrogels with bactericidal action have been prepared combining a poly-βCD, a dextran modified with alkyl-side chains, and a nitric oxide (NO) photodonor functionalized with an adamantyl group. Both alkyl-side chain and adamantyl group formed inclusion complexes with poly-βCD, and led to a network able to release NO on demand when exposed to visible light. The hydrogels were shown useful for photo-regulated killing of Gram-negative antibiotic-resistant bacteria.[143] βCD/adamantane interactions have also been exploited as intermediate linkers between the carrier and the therapeutic agent[60,144] or between a silicon nanowire substrate and gene-encapsulated nanoparticles for improved, serial transfection of cells that settle on the substrate.[145] Polyacrylate-based nanoparticles for selective delivery of doxorubicin to tumor cells have been prepared combining polyacrylates functionalized with adamantane and βCD, and decorated with folic acid. The nanoparticles showed excellent blood circulation and selective accumulation in tumor cells, being able to inhibit tumor growth *in vivo*.[146]

It is known that PEG makes the nanocarriers to be silent at the blood stream, but may hinder endosomal escape after cellular uptake. To solve this dilemma, several strategies involving

supramolecular assemblies have been tested. For example, redox-sensitive PEG-sheddable systems were designed using βCD cross-linked with polyethylenimine conjugate to MC11 peptide (host) and adamantyl-SS-PEG (guest). The supramolecular nanoparticles were shown able to form polyplexes with plasmid DNA, which were stabilized by the PEG chains in the extracellular environment.[147] Highly reductive intracellular environment facilitated the removal of PEG shell. The supramolecular polyplexes allowed tumor-targeted gene delivery *in vivo*, being more efficient than similarly prepared PEG-undetachable polyplexes.[147] Using other strategy, pH-sensitive PEG-removable supramolecular nanoparticles have been prepared combining ortho ester modified-βCD and adamantane-modified PEG.[148] The modified-βCD was prepared by means of a 'click reaction' between cyclic ketene acetals (CKAs) and the primary hydroxyl group 6-OH-βCD at different ratios molar. These derivatives (designed as CKA-CD) are too hydrophobic to dissolve in water.[148] Once adamantane-modified PEG is added to the medium, amphiphilic supramolecules are formed (Fig. 8). In the obtained architecture, most ortho ester linkages are located inside the hydrophobic core and a minority remains at or near the core/shell interface. The nanoparticles are stable at pH 8, but at pH 7.4 the ortho ester linkages located at the interface quickly hydrolyze, provoking the lost of PEG shell, which in turn triggers the aggregation of the hydrophobic inner cores.[148] The remnant ortho esters groups inside the cores are less exposed to the medium and hydrolyze little by little at neutral pH.[149]
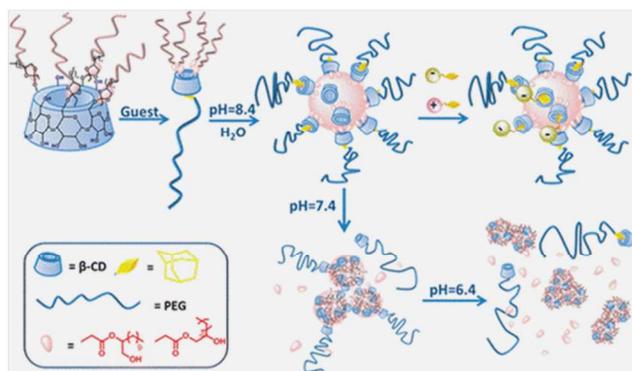

Fig. 8 Functionalizable and PEG-sheddable supramolecular nanoparticles programmed for pH-triggered dePEGylation and degradation. Reprinted with permission from Ji et al.[148] Copyright 2014 American Chemical Society.

Other guests such as benzimidazole, β-benzyl L-aspartate, alkyl chains, cholesterol and even hydrophobic drugs have been also shown suitable for creating zipper-like assemblies.[150-154] Nano-assembled carriers have been obtained via multiple host-guest complexes between a poly-CD and a polymer-paclitaxel conjugate (poly-PTX) (Fig. 9).[155] Poly-CDs spontaneously assembled with poly-PTX through multiple interactions, which lead to improved drug stability and solubility, compared with common PTX/βCD complexes.[156] The high stability of the assembled nanocarriers enables prolonged blood circulation time and avoids premature drug discharge, minimizing exposition of

normal tissue to the drug. Interestingly, anionic porphirins have been shown to form dimers with octaarginine-modified per-O-methyl-βCDs, and the resultant supramolecular particles exhibited enhanced intracellular delivery.[157]
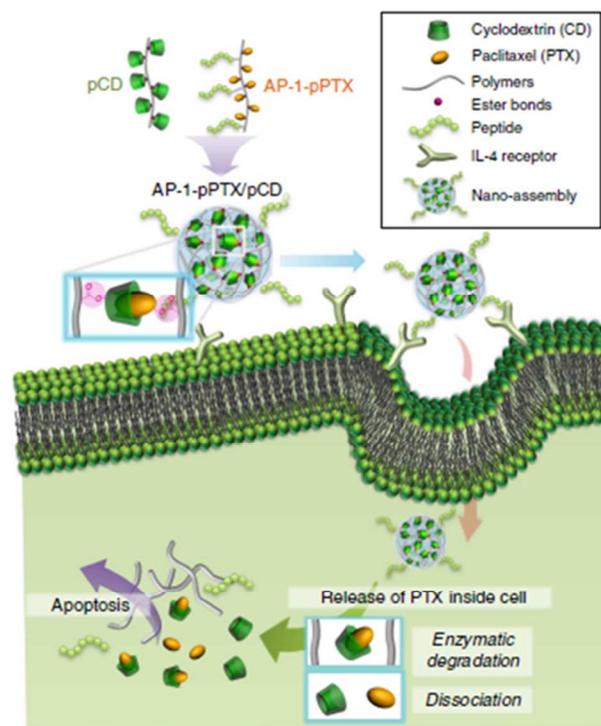

Fig. 9 Nanoparticles formed through interactions of poly-CD with polymer-paclitaxel conjugates (poly-PTX) are stable in blood stream and can be passively or actively targeted to cancer cells. Paclitaxel release is enzymatically triggered from the nano-assemblies and causes apoptosis. Reprinted from Namgung et al.[155] with permission from Elsevier.

For drugs that exhibit insufficient affinity for CD to trigger the zipper-like assembly, it is possible to synthesize drug-adamantane conjugates. For example, adamantyl-conjugated doxorubicin and paclitaxel have been shown to assemble with cationic polymers of βCDs cross-linked with polyethyleneimine.[70] The formed nanoparticles can also incorporate plasmid DNA or siRNA, for dual treatment of tumor cells.[158,159] Drug and adamantane are linked by a hydrazone bond, which can be cleaved at the acid pH of tumor cells. In other example, adamantane-doxorubicin prodrug has been encapsulated in pH-responsive capsules prepared by layer-by-layer assembly of polyaldehyde dextran-graft-adamantane and carboxymethyldextran-graft-βCD on CaCO₃ nanoparticles. Since adamantane was grafted to both doxorubicin and dextran though pH-cleavable hydrazone bonds, the supramolecular structure disintegrated under weak acid conditions and thus enabled site-specific drug release in tumor environment.[160] If active targeting is pursued, a similar strategy can be applied: the targeting group is conjugated to adamantane or adamantane-PEG. This approach has been already proved efficient for decoration of nanoparticles with RGD peptide for target delivery to $\alpha_v\beta_3$ integrins overexpressed on the surface of tumor cells.[161]

The host-guest mechanism allows incorporation of gold nanoclusters as part of the assembled structures. As an example, gold nanoparticles bearing adamantane moieties and CD-grafted hyaluronic acid form porous nanoaggregates that can host a variety of antitumor agents. Hyaluronic acid facilitated the endocytosis by tumor cells, and drug release was shown to be triggered by mild acidic pH.[162] Similarly superparamagnetic nanoparticles functionalized with adamantane groups can be incorporated to supramolecular aggregates of adamantanamine-grafted polyamidoamine dendrimers, βCD-grafted branched polyethyleneimine, and adamantane-PEG. These nanocarriers have been shown useful to magnetothermally responsive release of doxorubicin in a colorectal adenocarcinoma cell line.[163] Biodegradable graphene oxide (GO) nanostructures have been also integrated into supramolecular assemblies. βCD-functionalized GO (GO-CD) assembled with hyaluronate adamantane have been evaluated as water-dispersible nanocarriers for delivery of camptothecin to tumor tissues.[164] GO-CD prepared as uniform nanosheets can be degraded either extra- or intra-cellularly. Camptothecin loading occurred through π-stacking with GO (Fig. 10) and the resultant drug-loaded nanocarrier exhibited an excellent stability in both saline and serum environments and better anticancer activity toward breast cancer cells than the free drug.
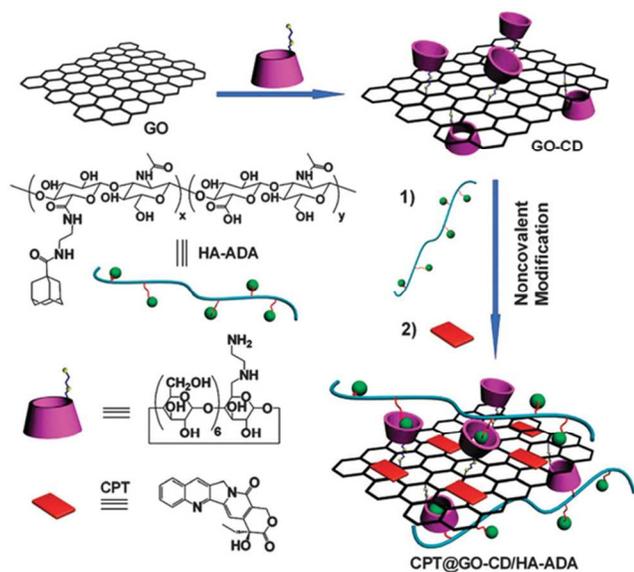

Fig. 10 Construction of a supramolecular nanocarrier for selective delivery of camptothecin toward malignant cells. β-CD functionalized graphene oxide (GO-CD) incorporated hyaluronated adamantane (HA-ADA) through host-guest assembly for specific targeting to hyaluronic acid receptor-expressing tumor cells. Camptothecin was loaded through π-stacking with GO. Reprinted from Zhang et al.[164] with permission from the Royal Society of Chemistry.

5. Micelles and vesicles

The ability of some hydophilic polymers to augment the solubilizing effect of CDs has been largely demonstrated.[165-167] Such synergism is generally attributed to formation of ternary aggregates or co-complexes between drug, CD, and polymer.[29,40,167] Polymeric micelles can be pointed out as versatile vehicles to increase oral bioavailability of drugs that exhibit poor solubility or permeability.[93] However, for many other micellar systems detrimental effects on solubilizing capability have been observed after the incorporation of CDs. The adverse effects are mostly related to the capability of CDs to host the hydrophobic chains of the surfactants and, thus, to hinder their participation in the micellar assembly.[168] As a consequence, the critical micellar concentration of the surfactant increases and the CDs involved in the hosting of the surfactant are not able to uptake drug molecules. This detrimental effect has been observed both for conventional surfactants and for amphiphilic block copolymers. For example, incorporation of Pluronic® F127 to drug solutions prepared using hydrophilic derivatives of βCD (e.g. hydroxypropyl-βCD (HPβCD) or methyl-βCD (MβCD)) leads to a competition for the cavity of CD that may cause the displacement of the drug as the polypseudorotaxane is formed.[89,169,170]

To overcome the above reported problems, different strategies to incorporate CDs into micellar systems are being explored. CDs are called to play three different roles: i) CDs reversibly form complexes with hydrophobic groups, which can be broken and re-formed as a function of a variety of stimuli;[13,171] ii) CDs act as host of macromolecular guests creating poly(pseudo)rotaxanes,[172,173] which can lead to supramolecular polymer micelles;[174] and iii) CDs can serve as starting cores to graft amphiphilic polymers and form star-shaped unimolecular micelles[175] or block copolymer micelles.[176]

Biodegradable βCD-conjugated amphiphilic copolymers were prepared combining poly(lactic acid) (PLA) and monomethoxy poly(ethylene glycol) (mPEG).[177] In aqueous solution, βCD-PLA-mPEG copolymer forms micelles of 173.4 nm. The size decreases to 159.2 nm after loading indomethacin in the hydrophobic cores mediated by inclusion complexes with βCD[171,176] Combination of βCD-PLA-mPEG with PLA-mPEG (3:7 or 8:2 molar ratio) led to more stable mixed micelles that released the drug at lower rate as the proportion in βCD-PLA-mPEG increased, which could be useful for prolonging circulation time.[178] Drug inclusion in the β-CD core of micelles efficiently minimized the burst effect as well as drug toxicity.

Supramolecular polymeric micelles have been prepared using maleic anhydride modified αCD (mah-αCD) and poly(ε-caprolactone) (PCL).[174] αCD and PCL initially assembled in THF/water to form an amphiphilic complex. Removal of THF resulted in a second assembly process in which the supramolecular amphiphilic polymer formed micelles with a mean diameter of 30 nm. These systems showed high prednisone acetate loading (39.5%) and biphasic release, with a rapid delivery in the initial stage and a subsequent gentle rate.

A pH-sensitive amphiphilic copolymer has been designed mixing a benzimidazole-functionalized PCL with a βCD-functionalized dextran.[62] The formed supramolecular PCL-*b*-dextran block copolymer self-assembled as micelles that were stable at pH 6.0-7.4, but dissociated at intracellular pH < 6.0; protonization of benzimidazole led to a great decrease in the binding constant with βCD, resulting in the dissociation of the complex. These supramolecular systems could efficiently load

and deliver doxorubicin into tumor cells, providing a powerful mean to release the drug at the tumor site.[62]

Amphiphilic CDs can be prepared with a variety of substituents in order to self-assemble forming micelles or vesicles.[179] Vesicles (100-150 nm) can be obtained by extrusion of αCD amphiphiles in water through a polycarbonate membrane. Compared to conventional liposomes, CD vesicles have the advantages of being capable of selective binding of hydrophobic guests and decorating with target molecules by simple mixing in aqueous medium. For example, vesicles of αCD amphiphiles can be decorated with bifunctional azobenzene-carbohydrate conjugates, by means of inclusion complexes with the transisomer of the azobenzene group. The carbohydrate moieties protruding from the vesicle can bind a variety of proteins, endowing the vesicles with transport capability. Light-controlled isomerization to the cis form makes the inclusion complexes to break, which in turn triggers protein release.[180]

Polycationic CDs have been shown to self-organize in the presence of DNA, forming compact CDplexes for safe delivery to cells.[181] Amphiphilic versions of these polycationic CDs may lead to improved ability to self-assemble and to cross cell-membranes.[23,182] Nanocomplexes of polycationic amphiphilic CD and pDNA that were subsequently decorated with folic acid exhibited superior ability to recognize tumor cells for cancer therapy.[183] Tetradecacationic amphiphilic derivatives of βCD, bearing 14 amino groups and 7 thioureido groups at the primary face and 14 hexanoyl chains at the secondary face, have been shown to form positively charged CDplexes that efficiently transfect several cell lines.[184] The CDplexes interacted ionically with folic acid to form ternary nanoparticles (Fol-CDplexes) capable of active targeting to cancer cells (Fig. 11). *In vivo* results indicated that Fol-CDplexes increase transfection activity in the lung and the liver compared to non-targeted CDplexes.[183]
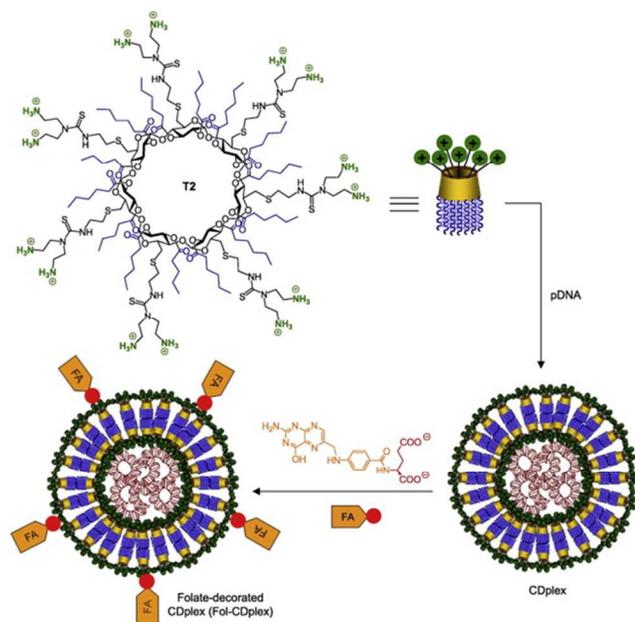

Fig. 11. Design of folate-decorated nanocarriers prepared combining a polycationic amphiphilic CD derivative (T2) with plasmid DNA and folic acid (FA) (Fol-CDplexes). Reprinted from Aranda et al.[183] with permission from Elsevier.

A poly-6-cationic amphiphilic cyclodextrin (CD) (heptakis[6-(2-amino-ethylthio)-6-deoxy-2-O-octylsulfanylpropyl]-β-cyclodextrin hepta-N-trifluoroacetate) has been investigated for transfection of undifferentiated and differentiated intestinal epithelial cells (Caco-2), resulting in high levels of reporter gene expression.[185,186] CD nanoparticles loaded with therapeutic siRNA (targeting tumour necrosis factor α) were administered via rectal route for specific delivery to macrophages, resulting in clinical improvements in a mouse model of acute colitis.[187] The arrangement of the lipid tails conjugated to the CD surface affected to the transfection efficiency of Caco-2 cells, the endocytosis pathway of the CDplexes, and their intracellular trafficking. Further studies to evaluate these CDplexes as candidates for gastrointestinal gene delivery revealed that the transfection efficiency was enhanced after incubation in bile salts, but was reduced after incubation in gastric and intestinal fluids and mucin.[186] Knowledge gained on the barriers posed by the gastrointestinal extracellular environment to gene delivery may help to optimize the CDplexes; for example, gastric instability could be overcome by microencapsulation and enteric coating or PEGylation.[186] Enhanced intestinal penetration could be afforded by conjugation of cell-penetrating peptides to the CDs, as observed for insulin.[188] PEI-CD-cholesterol micelles have been tested for single delivery of doxorubicin or siRNA targeting ABCB1 (multidrug resistance protein 1, MDR1) and for dual delivery of both drug and siRNA to tumor cells. The double layer structure obtained when combined with both therapeutic agents allowed an earlier release of siRNA for P-gp inactivation, followed by subsequent release of the drug once the efflux pump had been inactivated. Cell studies and in vivo experiments confirmed the synergistic therapeutic effects of co-delivering doxorubicin and siRNA to MDR tumor cells.[189]

6. Liposomes

Liposomes have been widely evaluated as drug nanocarriers due to their ability to encapsulate both hydrophilic and hydrophobic drugs and to modify their *in vivo* biodistribution. However, spontaneous entrapment efficiency is low and typically a pH-gradient between the inner and outer phases must be created for the loading of hydrophilic drugs. Many publications have shown that liposome loading depends on transmembrane pH gradient, lipid composition, internal buffering capacity, drug solubility and membrane-water partitioning, among other factors.[190-192] After *in vivo* administration, liposomes poorly control the release of lipophilic drugs.[193,194] These drawbacks have prompted the evaluation of CDs as tools able to regulate drug loading and release processes in liposomes.[195] Nevertheless, careful selection of CD type and complex preparation method is required for taking advantage of this combined approach.[196-198]

HP-βCD has higher aqueous solubility and more lipophilic interior than βCD, and thus can incorporate greater amounts of drug in vesicles,[195,199] if the amount of HP-βCD in liposomes do not cause destabilizing effects.[200] For example, mupirocin loading into PEGylated nanoliposomes strongly depended on HP-βCD concentration: at low concentrations, the loading was promoted,

but above 5% HP-βCD the loading was hindered. Incorporation of HP-βCD in the aqueous phase during liposomes formation notably enhanced drug loading and prevented premature release in serum.[201] On the other hand, methyl-βCDs have been shown to solubilize liposomes (i.e., transforming liposomes to micelles) as they form complexes with the lipids. For example, bioadhesive Carbopol® 974P gels containing stearylamine liposome-encapsulated inulin showed faster release after incorporation of methyl-βCD. Liposomes permeability varied as a function of methyl-βCD:lipid ratio.[202]

Piel et al.[203] showed the benefits of betamethasone-in-CD-in-liposome formulation, hosting the drug in the aqueous compartment and not in the lipid bilayer. HP-γCD and methyl-βCD form highly stable complexes with betamethasone ($K_{1:1}$ equals to 12,606 and 10,011 M^{-1}, respectively) which notably increases drug apparent solubility. Importantly, these CDs have low affinity for membrane lipids and therefore facilitate the encapsulation of highly concentrated drug solutions with minimal disturbance of lipid components at the liposome membrane. Double-loading techniques that involve liposome preparation with the plain drug incorporated into the lipophilic phase and its CD complex into the aqueous phase may provide formulations that have a rapid onset action and a prolonged effect.[204] Skin penetration of classical and highly deformable (elastic or ultraflexible) liposomes containing dexamethasone either in the aqueous compartment as inclusion complexes with HP-γCD or in the lipid bilayer has been recently compared.[205] In general, the presence of HP-γCD diminished the amount of drug in the epidermis due to the reservoir effect.

Other strategy to improve the encapsulation efficiency of nonpolar, hydrophobic drugs that cannot benefit from active loading (impelled by a transmembrane pH gradient) is the inclusion complex formation with CDs bearing weakly basic or acidic ionizable groups.[191] The external pH is regulated so the complex is non-ionized and thus can penetrate the lipid bilayer. Inside the liposome, the CD outer groups become ionized and the complex remain trapped (Fig. 12). This approach has been shown very useful for formulating chemotherapeutic agents that require tumor specific release.
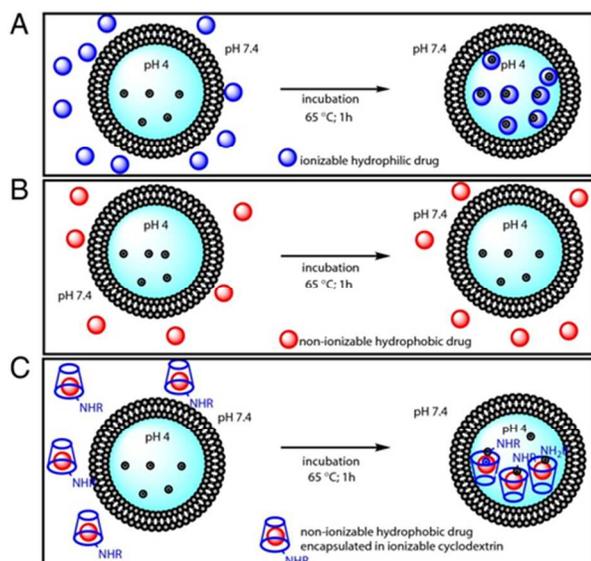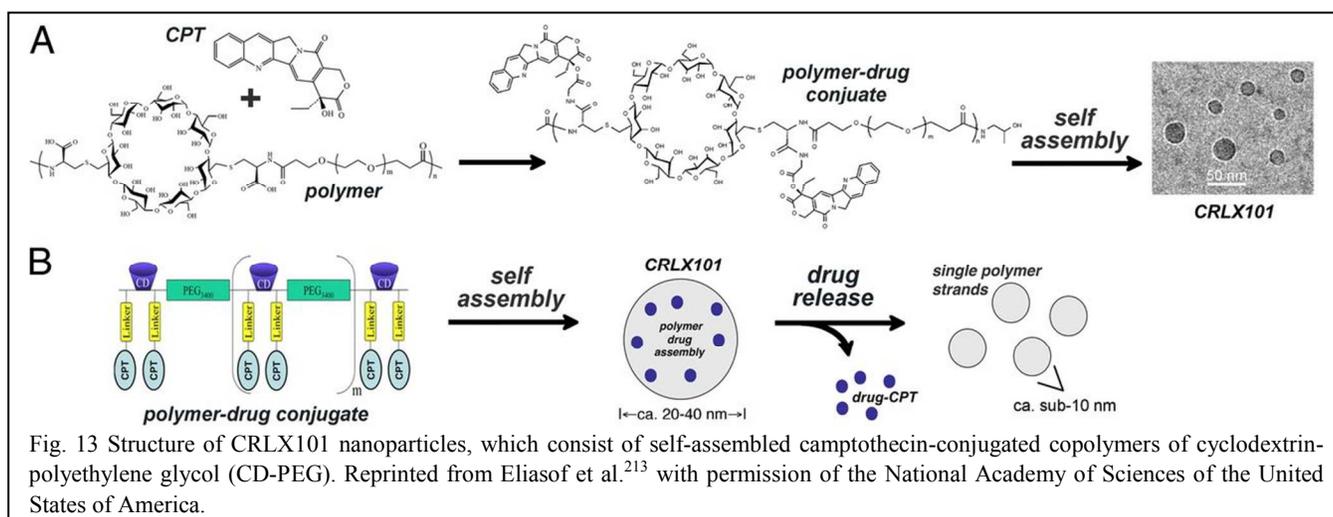

Fig. 12 Transmembrane pH gradient (active loading) is useful for the encapsulation of (A) ionizable hydrophilic drugs or (C) poorly soluble drugs forming complexes with an ionizable cyclodextrin (R = H, ionizable alkyl or aryl groups). Oppositely, (B) poorly soluble hydrophobic drugs are minimally incorporated in the absence of ionizable cyclodextrin. Reprinted from Sur et al.[191] with permission of the National Academy of Sciences of the United States of America.

7. Supramolecular cyclodextrins in clinical trials

As mentioned above, CD-based supramolecular assemblies can protect therapeutic molecules from enzymatic and chemical degradation, target the cargo to specific cells and release it at the adequate rate. Cell assays and *in vivo* studies evidenced that CD-based nanoparticles can be taken up by a variety of cells, including those of tumors and immune system.[206,207] Two formulations based on CD supramolecular assemblies are currently under clinical trials: CRLX101 (formerly IT-101) and CALAA-01.[208]

Self-assembled nanoparticles based on camptothecin conjugated to a linear cyclodextrin-PEG copolymer (CRLX101) are in Phase 2 studies as therapy for small-cell lung cancer and ovarian cancer.[209-211] CRLX101 consist of a linear backbone having repeating units of βCD and PEG with pendant moieties for linkage (through a labile ester bond) of camptothecin in its active form (10-12 wt.% loading) (Fig. 13). Once conjugated, the drug can be also hosted inside the CD units of the same or other polymer chains. The copolymer self-assembles into highly reproducible nanoparticles (20- to 30-nm) that increase camptothecin apparent solubility more than 1000-fold compared to free drug.[212] The ester linkage prevents premature ring opening and facilitates controlled release inside tumor cells (Fig. 13). CRLX101 have been shown able to overcome the bioavailability and safety limitations of camptothecin and to notably improve drug therapeutic efficacy.[213] Notably greater drug levels in tumor compared to plasma and other organs is explained by the enhanced permeation and retention (EPR) effect, which facilitates the accumulation into solid tumors and localized drug release for several days. Pharmacokinetic studies of CRLX101 in diverse animal models and humans revealed that the area under the curve correlates with milligrams of drug per square meter for all species. Once camptothecin is released, the nanoparticles disassemble and the short polymer chains are cleared through the kidney.[213] Combining CRLX101 with a common antiangiogenic drug may offer a novel way to address cancers that develop resistance to antiangiogenic drugs and radiation therapy. Other drugs formulated in the same platform (CRLX301) are expected to enter clinical trials by the end of 2014.

Fig. 13 Structure of CRLX101 nanoparticles, which consist of self-assembled camptothecin-conjugated copolymers of cyclodextrin-polyethylene glycol (CD-PEG). Reprinted from Eliasof et al.[213] with permission of the National Academy of Sciences of the United States of America.

CALAA-01 is a nanoparticle (50-70 nm) formulation of siRNA that consists of a CD-polycation, adamantane (AD)-PEG conjugate (PEG MW of 5000) and AD-PEG-transferrin as targeting ligand, to be administered intravenously for the treatment of solid tumors.[214] The short CD-polycation is required for encapsulation of siRNA, forming CDplexes (Fig. 14). CDs remaining at the surface of the nanoparticles are used for host-guest interaction with AD-PEG for steric stabilization, and with AD-PEG-transferrin for efficient targeting. The PEG spacer facilitates the binding of the targeting agent to cell surface receptors, which enables CALAA-01 endocytosis. The nanoparticles accumulate in tumors in amounts that scale linearly with dose levels given to the patients and demonstrate gene inhibition by RNAi.[215] Results from a human phase Ia/Ib clinical trial indicate that CALAA-01 causes minimal liver and kidney toxicity, and provides target delivery of siRNA.[216]
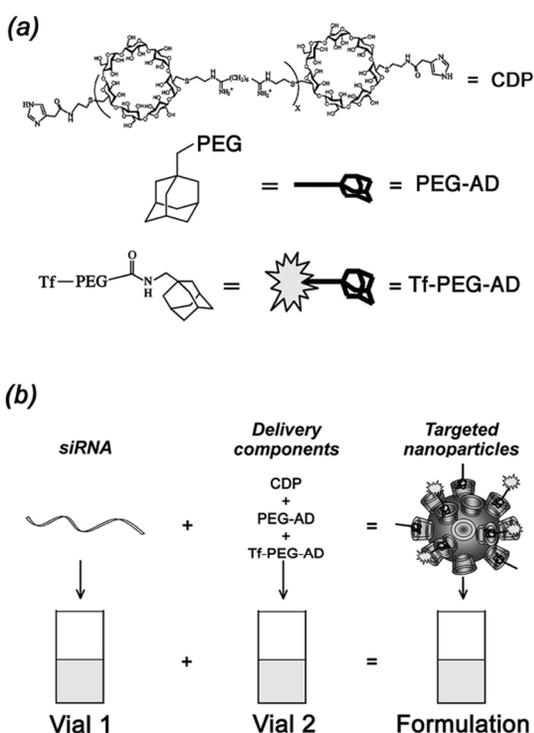

Fig. 14. Composition and structure CALAA-01 nanoparticles incorporating siRNA. The formulation consists in one vial with siRNA and other vial with a hydrosoluble cyclodextrin polymer (CDP), an adamantane-PEG conjugate (PEG-AD) and transferrin-PEG-AD. Before administration, the two vials are mixed together and the nanoparticles are formed via self-assembly of the four components. Reprinted with permission from Davis[214]. Copyright 2009 American Chemical Society.

8. Conclusions and a view to the future

Versatility of CD structure regarding both inclusion complex capability and functionalization of the OH groups opens wide, and still not fully explored, possibilities of creating supramolecular structures. Achievements accomplished so far clearly demonstrate the suitability of CD-based supramolecular assemblies, in the form of individualized nanocarriers or as colloids-associated gels, for facing up to diverse therapeutic demands. Efforts in the field have multiplied exponentially as demonstrated not only by the number of publications, but mainly by the detailed in vitro and in vivo characterization of the obtained structures. The already long experience in the use of individualized CDs as components of medicines is undoubtedly paving the way to the clinical trials of some developed prototypes, which have already provided results that clearly demonstrate the advantages they may offer in therapeutics. Nevertheless, deeper insight into efficacy but also safety in human beings is still required; knowledge about stability of the supramolecular entities after administration through a systemic route, performance regarding drug targeting and release, and subsequent clearance is mandatory for the correct design and further optimization of the CD-based carriers.

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a Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.
b Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.
5 *c Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain. Fax: 34 981547148; Tel: 34 881815239; E-mail: Carmen.alvarez.lorenzo@usc.es*
d Center of Experimental Orthopaedics, Saarland University,
10 *Homburg/Saar, Germany.*

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Table 1. Some recent examples of poly-CDs performance for drug and gene delivery.

Natural CD/ cross-linker/ polymer	Drug/ guest	Nanocarrier/ formulation	Outcomes	References
αCD/EPI	Vancomycin / PEO	Supramolecular gel of poly-αCD with PEO-based polymers	3D-poly(pseudo)rotaxane assemblies suitable as syringeable systems for sustained drug release.	[59]
αCD/OEI polymers	pDNA	Cationic polyplexes	αCD-OEI star polymers showed pDNA binding capability leading to nanoparticles that had high gene transfection efficiency.	[133]
βCD/ EPI	Bupivacaine hydrochloride	Mucoadhesive formulation	PolyβCDs as a tool for tailoring drug release rate from a buccal drug delivery system.	[129]
βCD/EPI/hydrox yapatite	Gentamicin and cisplatin	Bioceramic scaffold	Poly-βCD incorporated into a bone regeneration scaffold enhanced drug loading and sustained drug release.	[121]
βCD/ EPI	Glipizide	Controlled drug delivery system	Enhanced oral bioavailability of glipizide.	[55]
βCD/ EPI	Hypericin	Controlled drug delivery system	Improved physical and chemical features of hypericin formulation.	[127]
βCD or carboxymethylat hed βCD / EPI	Ketoprofen	Drug-in polyCD-in nanostructured lipid carriers	Enhanced solubility and permeability.	[122]
βCD/ EPI / choline chloride (CC)	Indomethacin	Supramolecular complexes	Improved drug loading and controlled release in alginate hydrogel.	[132]
βCD/ EPI/ CC	Insulin	Supramolecular complexes	Improved gastric stability and controlled release when incorporated into alginate/chitosan nanospheres.	[123, 124]
βCD/ EPI	Methyl orange	Microgel	pH-responsive release of methyl orange.	[125]
βCD/ EPI / CC	Triclosan	Solution	Enhanced solubility and dissolution rate.	[128]