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Cyclodextrin-based rotaxanes as a versatile platform for biological and medicinal applications

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Mechanically interlocked molecules (MIMs) such as rotaxanes and catenanes attract significant interest due to their unique structures and dynamic properties. Cyclodextrin-based rotaxanes (CD-rotaxanes) have emerged as promising supramolecular systems for biological and medicinal applications. Their host-guest interactions and mechanical bonds provide enhanced stability, stimuli-responsiveness, and tunable functionality. This review highlights their roles in targeted therapy, controlling drug release, theranostic agents, enzyme inhibitor, gene transport and bioimaging. Challenges and future perspectives in translating CD-rotaxanes to biomedical applications are discussed, emphasizing their potential as a next-generation therapeutic platform.

In 2025, the fact that mechanically interlocked molecules (MIMs) are no longer considered as a new and fundamental research area with limited applications is rising among the scientific community. This can be attributed to their rapid evolution from mere synthetic curiosities to functional materials with practical applications in diverse fields, including artificial molecular machines (2016 Nobel prize in Chemistry)[1–4], smart materials[5], catalysis[6], molecular recognition and sensing[7], among others. MIMs comprise two or more components linked together, which cannot be separated without breaking one or more chemical bonds; the components are thus physically interlocked rather than covalently bonded[8]. Rotaxanes, where a macrocycle encircling an axle is prevented from dissociating by sterically bulky end-groups, and catenanes, in which two or more macrocycles are linked in a topological manner, are the classic examples of MIMs[3,5]. A defining feature of rotaxanes is the mechanical bond, which arises from the topological entanglement of the macrocycle and the axle. Unlike covalent bonds, mechanical bonds do not involve direct sharing of electrons. Instead, the macrocycle is physically trapped around the axle due to the presence of bulky end-groups that prevent dethreading. This mechanical interlocking imparts unique dynamic properties and stimuli-responsiveness to rotaxanes (Fig. 1A).

MIMs especially rotaxanes exhibit a promising candidate that can be used in biological and medicinal studies as a new generation of bioactive compounds[9,10]. The mechanical bond provides three main properties. First, multiple functionalities are obtained in a well-defined spatial arrangement in a relatively low synthetic effort, making MIMs valuable for applications in biosensing, targeted drug delivery, and biomolecular imaging. Second, the flexibility of the mechanical bond affords controlled molecular motion and conformational changes enabling response to specific biological stimuli in a precise and reversible way. Third, the mechanical bond can protect sensitive functional groups by encapsulating them within the structure. This is particularly valuable for drug delivery applications, where therapeutic agents are shielded from degradation in biological environments.

Interestingly, MIMs have been recognized in nature for a long time ago[11]. Catenated DNA in which circular mitochondrial DNA molecules are linked as links in a chain were identified in living cell extracts back in 1967[12]. Lasso peptides that are sometimes referred to as [1]rotaxanes were found in 1992[13]. The unique structure of these peptides consists of a peptide tail threaded through a macrolactam ring, creating a structure resembling a lariat. Inspired by these observations, many researchers worldwide have developed novel MIMs architectures and demonstrated their functions in medicinal and biological studies, such as prodrug[14], biosensing[7,15], and drug delivery[16]. Consequently, Papot[9], Niemeyer[10], and Schaufelberger[17] has recently reviewed the recent advances in the development of MIMs and their use in biological and medicinal related studies. However, these comprehensive reviews focused on the synthesis of the interlocked architectures and the role of the mechanical bond in the potential application.

While there has been impressive progress in the field, cyclodextrin (CD)-based MIMs are a promising candidate to fulfill the requirements of biocompatible molecules. Although the general topic was reviewed in the literature, no particular emphasis was given to research on CD-based rotaxanes and their application in biological studies. This review provides an overview of the current applications of CD-based rotaxanes in biological systems. Specifically, we highlight the use of these architectures for targeting drugs, medical imaging, controlling drug release, enzyme inhibition, transport of biomolecules, and theranostic agents.

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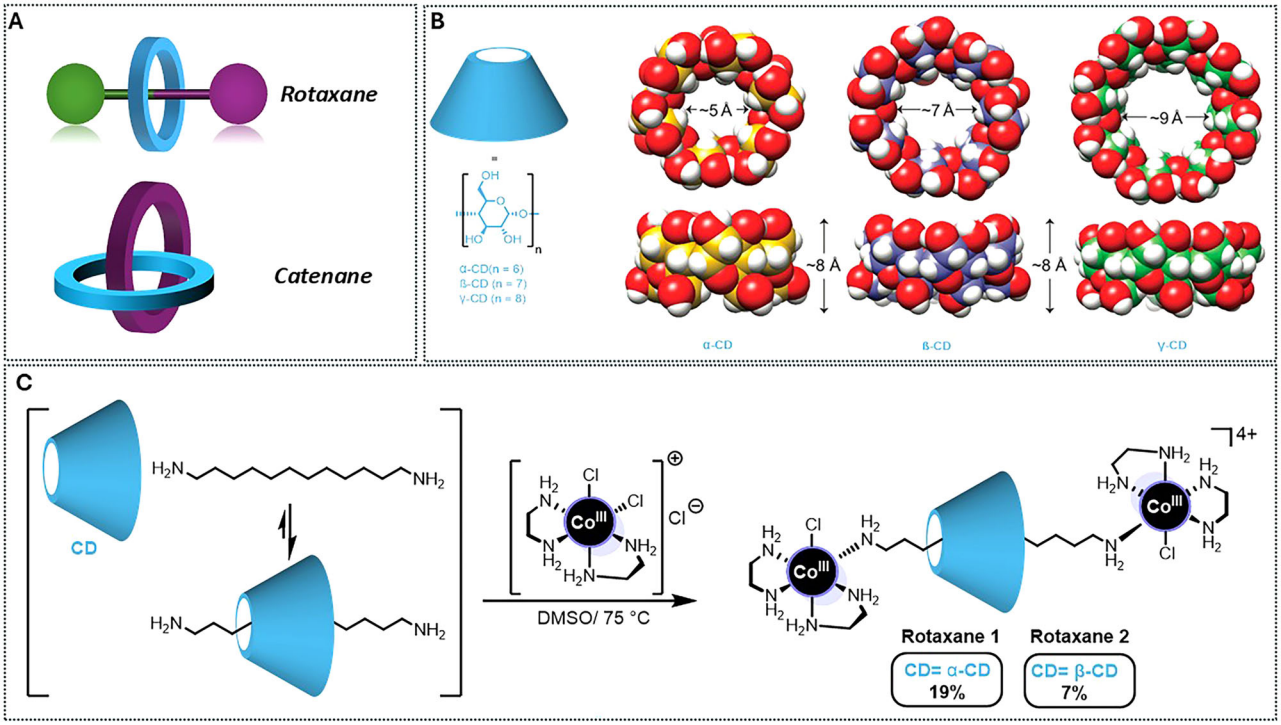

Fig. 1 | Overview of CD-based interlocked molecules and Ogino's pioneering rotaxane synthesis. A Schematic representations of a rotaxane (top) and a catenane (bottom). **B** Structural, space-filling, and graphical representations of the three main CD molecules: α-CD, β-CD, and γ-CD. **C** Ogino's seminal template-directed synthesis of rotaxanes **1** and **2** from diaminododecane and α-CD or β-CD, respectively.

Main text

CD macrocycles

CD macrocycles are a family of cyclic oligosaccharides, consisting of a macrocyclic ring of glucose subunits joined by α-1,4-glycosidic bonds first isolated from starch-fermenting bacteria cultures as mysterious crystalline substances by Villiers in 1891[18]. CDs have been intensively studied due to their abilities to act as hosts. In particular, CDs have a hydrophilic exterior and a hydrophobic interior that promote the formation of inclusion complexes via aggregation of the hydrophobic interior and hydrophobic guest molecules. Consciously, CDs became one of the most commonly utilized hosts in chemistry due to their affordability, high stability, solubility in water, biocompatibility, biodegradability, and, importantly, their ability to encapsulate a huge number of guest molecules with varying association strengths[19]. Three commonly used CDs are α-CD (six glucose units), β-CD (seven glucose units), and γ-CD (eight glucose subunits) (Fig. 1B). Though larger CDs (such as δ, ε, ζ, η, etc.) are relatively rare[20], they have been known for some time and structurally characterized up to at least the 26-mer, φ-CD[21]. The cavities of CDs measure nearly 8 Å in depth (Fig. 1B), with diameters ranging from approximately 5 to 9 Å ($\alpha < \beta < \gamma$). There are thousands of known guest molecules that associate with CDs in water[22,23]. Generally, the association constants rise with increasing chain length, assuming the guests remain sufficiently soluble in water. Thus, and since water is the optimal solvent for host-guest interactions in CDs, so the need for aqueous conditions in synthesizing CD-based rotaxanes limits the range of applicable reactions. To overcome this limitation, one approach is to make CDs soluble in organic solvents by protecting some or all of their hydroxyl groups[24]. A common modification is partial or complete methylation[25]. Interestingly, despite their increased hydrophobicity, permethylated CDs exhibit greater water solubility than their unmodified counterparts. This suggests that hydrogen bonding between the hydroxyl groups is a key factor in the lower aqueous solubility of native CDs.

CD-based rotaxanes

CDs are suitable macrocycles for the formation of rotaxanes and there are countless examples of this. These are usually formed through the capping of the encapsulated guest molecule by bulky end groups to afford a [2]rotaxane. However, there are many examples of higher order rotaxanes also being formed. The first CD rotaxane was reported in 1981 by Ogino[26]. Ogino coupled α,ω-diaminoalkanes with two equivalents of a bulky cobalt complex through coordination bonding in DMSO in the presence of α-CD or β-CD to form the corresponding CD-rotaxane **1** and **2**, respectively. (Fig. 1C) Since then, many other CD rotaxanes have been formed using the capping technique with various other end groups. Today, CD-based rotaxanes have several applications in molecular shuttles[27,28], molecular ratchets[29–32], and molecular muscles[33]. CD can also be used for polyrotaxanes[34–38], and polypsuedorotaxanes along with functional materials[39]. Currently, CD rotaxanes have only one commercial application. A Japanese company—ASM—has developed polyrotaxane derivatives that can be used in coatings, adhesives, sealants, and elastomers. CDs are also used within medicine, being an ingredient within more than 30 approved drugs[40]. They can form complexes with biologically active hydrophobic compounds and help increase their water-solubility. This then allows them to be released under specific conditions. Furthermore, each of α-, β-, and γ-CD are generally recognized as safe by the United States Food and Drug Administration.

CD-based rotaxanes and their applications

CD-based rotaxanes as targeting drugs

Autophagy plays a crucial role in various diseases, and its induction is considered a potential therapeutic approach[41]. Several studies have shown that β-CDs can destabilize organelle membranes and induce autophagy[42]. Based on this, Nishida et al. have recently employed the polyrotaxane scaffold to regulate the cholesterol-binding ability of methylated β-CDs, thereby influencing their capacity to induce autophagy[43,44]. In this study, the authors hypothesized that methylated-β-CDs released from the polyrotaxane in acidic conditions like lysosomes could interact with organelle membranes and trigger autophagy. This approach aimed at overcoming

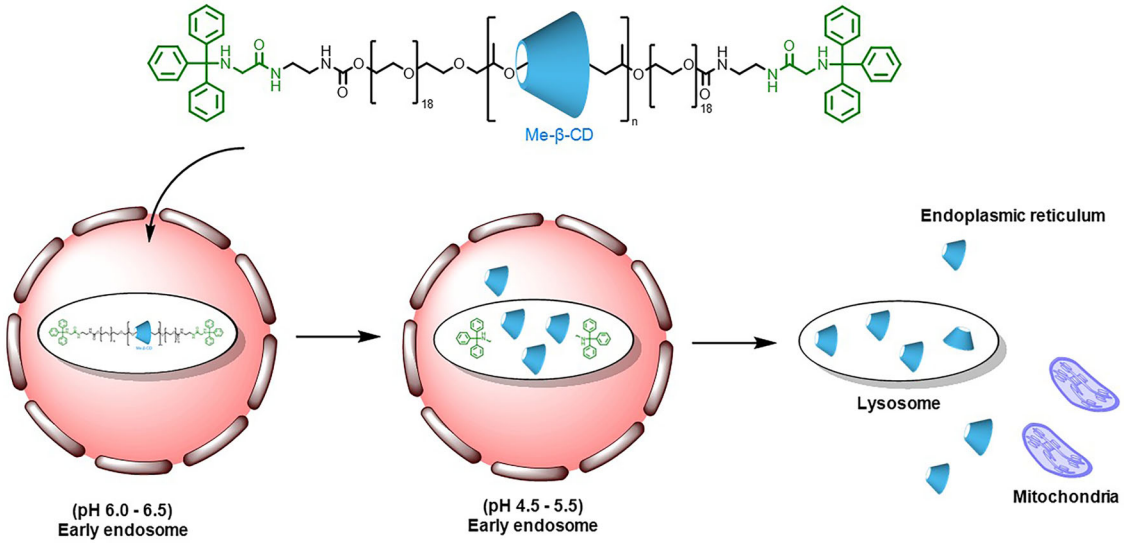

Fig. 2 | Acid-triggered disassembly of Me-β-CD. Schematic illustration of methylated β-CDs-threaded acid-labile polyrotaxane, which can dissociate to release threaded methylated-β-CDs in acidic compartments, and the subsequent stress induction in cellular organelles via the interaction with released methylated-β-CDs.

limitations of free β-CD derivatives, which tend to interact primarily with the plasma membrane. The results clearly showed that polyrotaxane preferentially accumulated in the endoplasmic reticulum and caused endoplasmic reticulum stress, confirmed by gene expression analysis and endoplasmic reticulum stress marker proteins. This endoplasmic reticulum stress led to autophagy induction, which was not observed with non-labile polyrotaxane, other modified polyrotaxanes, or the free methylated-β-CD (Fig. 2). Interestingly, solution-phase studies showed that methylated-β-CD polyrotaxanes exhibit excellent stability under physiological conditions (pH 7.4, 37 °C), with no degradation observed up to 48 h. Furthermore, polyrotaxane treatment induced autophagic cell death, even in apoptosis-resistant cells. The acid-labile design of polyrotaxane allowed for targeted release of methylated-β-CDs in acidic cellular compartments, leading to specific interactions with organelle membranes, particularly the endoplasmic reticulum. The role of the methylated-β-CD-polyrotaxane was crucial for the observed activity.

This study demonstrates that CD-polyrotaxane induces endoplasmic reticulum stress-mediated autophagic cell death, making it a promising candidate for treating apoptosis-resistant malignant tumors. This approach highlights the potential of using CD-polyrotaxanes as a novel strategy for inducing autophagy and autophagic cell death in therapeutic applications.

CD-based rotaxane nanovalves for controlling drug release

Stimuli-responsive materials are key compounds for the development of advanced technologies in drug delivery, sensors, and smart materials[45], as these systems can adapt to environmental changes and perform precise, controlled functions. Rotaxanes based on mesoporous silica nanoparticles (MSNPs) have been proved to be useful as stimuli-responsive systems due it their unique properties[46] (Fig. 3A). Consequently, Gayam et al. have developed an NAD(P)H:quinone oxidoreductase 1 (NQO1) enzyme-responsive MSNPs[47] based on α-CD-based rotaxane for in vivo tumor targeted delivery of doxorubicin anti-cancer drugs[48]. The reported drug delivery system is programmed to respond to NQO1 that is overexpressed in many cancer cells (e.g., A549), making it an ideal trigger for targeted drug delivery[49]. This rotaxane aims mainly to address challenges in developing smart drug delivery systems and improve the drug release mechanism. In the reported system, a benzoquinone stopper holds mechanically bonded α-CD close to the surface of the MSNP, preventing cargo release until NQO1 and NADH are both present (Fig. 3B).

The system showed zero release of the drug prior to exposure to NQO1 and NADH, which cleaved the benzoquinone stopper and allowed the CD to dethread and release the drug. In vitro and in vivo studies demonstrated efficient drug loading, controlled release, cellular uptake, and selective cytotoxicity. To the aim of this review, we emphasize that the α-CD macrocycle played a fundamental role in this promising activity by acting as a gatekeeper to prevent premature drug release. This MIM design provided precise control over the drug release mechanism, enabling targeted delivery specifically to NQO1-overexpressing cancer cells. The authors highlighted that this biocompatible and enzyme-responsive MSNPs rotaxane shows promise as a theranostic platform for targeted cancer therapy and can be a promising candidate for smart drug delivery systems. Notable, there are a few more promising studies based on stimuli-responsive CD-MSNP rotaxanes and we refer readers to more comprehensive sources for obtaining information on these studies[50–53].

In the studies presented above, the drug release process is irreversible; however, reversible and controlled drug release processes are favorable for various applications. Consequently, reversible rotaxane nanovalves have been developed[54]. In these systems, the macrocycle is designed to shuttle away from the surface, opening the pores and allowing the stored materials to be released. However, when the initial stimulus is removed or a second stimulus has been applied, the macrocycle shuttles back to its original station. These reversible and controlled processes offer additional benefits that help to avoid toxic side effects caused by complete release[55].

Yan et al. have reported light-responsive CD-based rotaxanes for in vivo photothermal-controlled drug release[56]. This clever design features a molecular "gatekeeper" consisting of an α-CD threaded along a linear azobenzene-containing axle. The de-threading of α-CD was prevented by using a sulfonated naphthalene stopper that also was used to increase the water solubility of the nanoparticles (Fig. 3C). When exposed to 37 °C or visible light, the azobenzene isomerize from its *cis* to *trans* conformation, resulting the α-CD to shuttle away from the nanoparticle surface and "opening" the pore to allow curcumin release. Conversely, when irradiated with 365 nm light, the azobenzene reverts to its *cis* isomer, which increases the steric hindrance, causing the α-CD to shuttle back to its original pore-blocking position and prevent the curcumin release. The in vivo photothermal drug release was evaluated on zebrafish larvae as a model organism. When the larvae were kept in dark conditions at 24 °C for an hour, the overall curcumin fluorescence decreased by 11.5%. This reduction was attributed to the in vivo metabolism of released curcumin within the organism. In contrast, exposing the larvae to visible light at the same temperature for an hour resulted in a more significant 34.9% decrease in fluorescence intensity. When the temperature was raised to 37 °C in dark

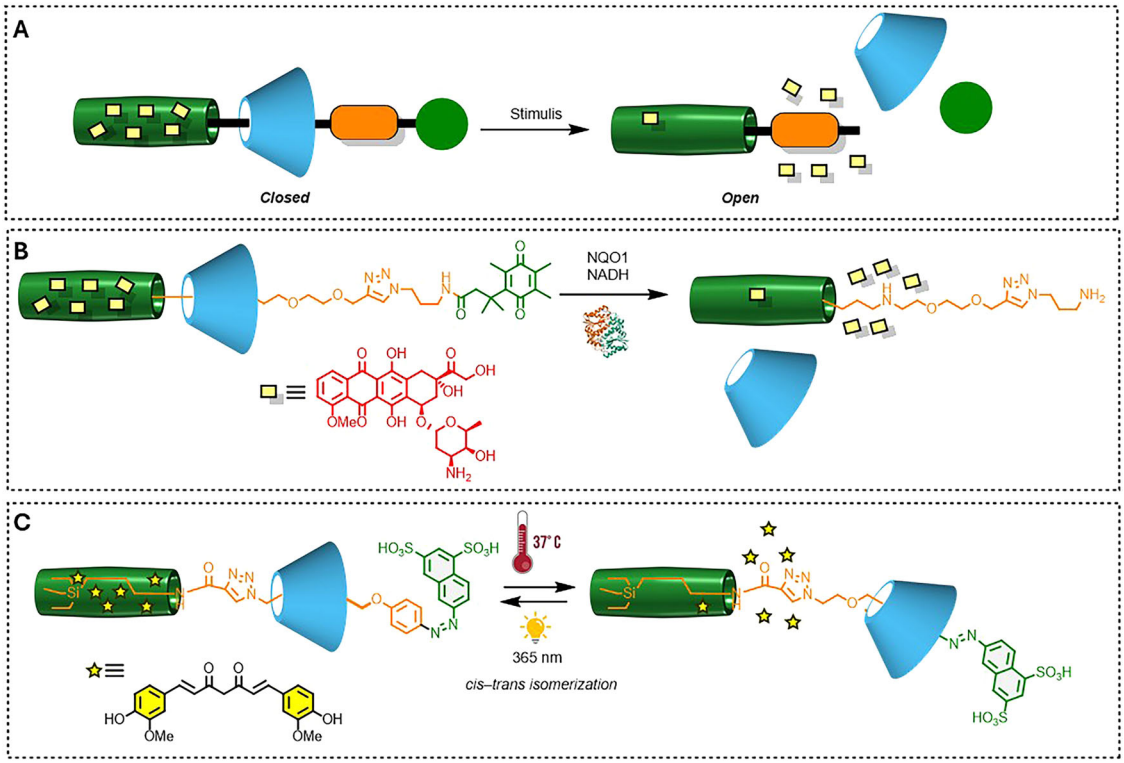

Fig. 3 | Stimuli-responsive MSNP-based rotaxane nanovalves. A Stimuli-responsive cargo release from MSNP-functionalized nanovalves. **B** NQO1 enzyme-responsive release of doxorubicin from MSNP-based rotaxane. **C** CD-based rotaxanes functionalized with azobenzene as reversible gatekeepers in MSNPs.

conditions, an even greater reduction of 45.9% was observed after an hour. Furthermore, since curcumin is known as a promising candidate for heart failure treatment, this study demonstrated that encapsulating curcumin in MSNPs significantly enhanced its therapeutic efficacy. When administered to zebrafish embryos with heart failure, the curcumin-loaded MSNPs successfully restored normal cardiac function. Interestingly, this promising technology was adapted later to prepare a CD-based rotaxane as a near infrared-triggered drug release for in vivo evaluation[57]. Compared to traditional drug delivery systems such as liposomes, micelles, and polymeric nanoparticles, CD-rotaxanes offer several distinct advantages. First, the mechanical bond in CD-rotaxanes enhances the stability of the drug carrier, preventing premature drug release and improving circulation time[58]. Second, the CD cavity can be functionalized with targeting ligands, such as antibodies or peptides, to improve targeting efficiency and reduce off-target effects[59]. Third, the unique structure of CD-rotaxanes can allow for higher drug loading capacities compared to other systems, particularly for hydrophobic drugs[59]. Finally, CD-rotaxanes can be designed to respond to specific stimuli, such as pH or enzymes, allowing for controlled drug release at the desired site of action[60].

CD-rotaxanes as theranostic agents

Theranostic agents are a fascinating and emerging area in medicinal chemistry. They constitute systems that are designed to provide simultaneously diagnostic and therapeutic benefits within a single molecular scaffold[61,62]. This integration offers several important benefits, such as personalized medicine, early detection, and precise treatment. Indeed, recent clinical achievements and the approval of Lutathera™ for somatostatin receptor-positive neuroendocrine tumors[63] and Pluvicto™ for prostate cancer targeting prostate-specific membrane antigen (PSMA)[64] underscore the increasing academic and commercial enthusiasm for theranostic agents as promising cutting-edge therapies. However, the field is far from developed, and the demand to integrate a new molecular scaffold with unique properties, such as those provided by MIMs to control drug delivery and controlled release[46,65,66], is increasing.

For this end, Yu et al. have recently reported on the potential of CD-based polyrotaxanes for cancer theranostics, showcasing their ability to combine chemotherapy and photothermal therapy effectively. The unique topological structure and stimuli-responsiveness of the polyrotaxane, particularly the role of β-CD in controlled drug release and crosslinking, contribute to the enhanced anti-tumor performance and reduced toxicity of the shell-crosslinked nanoparticles[67]. In their study, they used an amino-functionalized β-CD-NH_2 macrocycle to prepare a polyrotaxane that utilizes an amphiphilic copolymer as its axle. This axle consists of a hydrophobic polycaprolactone stoppered with a perylene diimide photosensitizer on one side and a hydrophilic polyethylene glycol stoppered with a cyclic pentapeptide "cRGDfK" targeting on the other side (Fig. 4A). The cRGDfK is used mainly for the delivery enabling the nanoparticles to bind to $\alpha_v\beta_3$ integrin receptors overexpressed in tumors.

The reported polyrotaxanes assemble into core-shell nanoparticles, ranging from 50 to 100 nm. The β-CD-NH_2 is crosslinked using a disulfide-containing diester, creating stabilized nanoparticles. These nanoparticles can encapsulate paclitaxel within their hydrophobic core through non-covalent interactions. The disulfide crosslinks are designed to break down in the presence of high glutathione concentrations within cancer cells. This breakdown destabilizes the nanoparticles, leading to selective drug release inside the cells. The rotaxane structure prevents crystallization of the polycaprolactone chains, resulting in high drug loading capacity and increased particle stability in buffer compared to nanoparticles made with only free axles. Furthermore, the perylenediimide stopper enables photothermal sensitization, converting laser irradiation into heat, inducing cell damage specifically in the targeted area. The cRGDfK stoppers effectively target cancer cells overexpressing integrin $\alpha_v\beta_3$, as demonstrated by in vitro studies where dye-loaded particles showed high fluorescence intensity within the cytoplasm of cells. Pre-treatment with free cRGDfK reduced fluorescence intensity, suggesting receptor-mediated endocytosis as the uptake mechanism. In vivo studies using HeLa tumor-bearing mice resulted in a 100% survival rate and complete tumor disappearance without recurrence when treated with both chemotherapy and photothermal therapy.

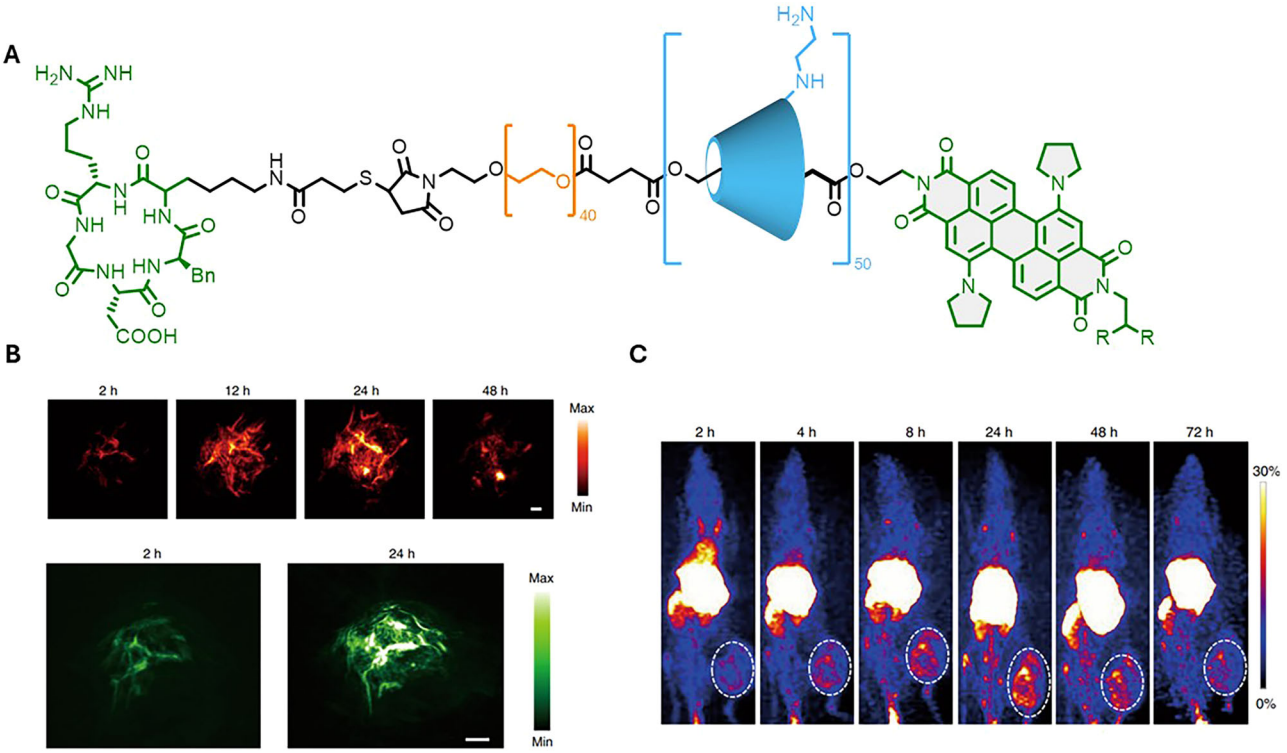

Fig. 4 | β-CD-based polyrotaxane nanoparticles for in vivo tumor imaging. A β-CD-NH_2-based polyrotaxane. **B** Representative photoacoustic maximum imaging projection and 3D images of the tumor in a living mouse after systemic administration of shell-crosslinked nanoparticles through i.v. injection. Scale bar is 2 mm. **C** Decay-corrected whole-body coronal PET images of HeLa tumor-bearing mice at 2, 4, 8, 24, 48, and 72 h after i.v. injection of 150 μCi of ^{64}Cu nanoparticles.

Histological analysis revealed no systemic toxicity. When tested on mice with aggressive 4T1 breast cancer, the combined chemo-photothermal therapy group showed the highest median survival rate, along with significant reductions in tumor weight and lung metastasis, highlighting the synergistic benefits of this approach. This study demonstrated that CD-based polyrotaxanes can provide a transformative solution to drug delivery challenges, achieving controlled and targeted drug release with enhanced therapeutic efficacy. By integrating photothermal therapy and chemotherapy, polyrotaxane-based nanoparticles present a versatile and promising platform for cancer treatment, demonstrating significant tumor regression and prevention of metastasis.

CD-rotaxanes as an enzyme inhibitor

Enzyme inhibition strategies using covalently immobilized ligand polymers[68] have shown promise in modulating enzymatic activity but often face challenges due to steric crowding, which becomes thermodynamically unfavorable for effective enzyme binding[69]. To overcome these limitations, polyrotaxanes containing a macrocycle conjugated to inhibitor ligands have been investigated[70,71]. These architectures capitalize on the high molecular mobility of polyrotaxanes, as they can freely rotate and slide along the axle. The resulting mobile inhibitors in polyrotaxane systems can adapt more effectively to enzyme surfaces, enabling simultaneous interaction with multiple binding pockets or allosteric sites. This adaptability enhances the cooperative effect of inhibitor–enzyme interactions, resulting in more potent and selective enzyme inhibition[72] (Fig. 5A). This area of research was primarily driven by Nobuhiko Yui and co-workers[73–76]. The first study showed that the human peptide transporter can be effectively inhibited using a modified CD-based polyrotaxane system[77]. This system consists of a polyethylene glycol axle with phenylalanine stoppers and α-CD conjugated with Val-Lys dipeptides (Fig. 5B). The target peptide is a crucial intestinal transport protein, is responsible for the absorption of dipeptide and tripeptide during the digestive process. In vitro experiments demonstrated that the reported α-CD Val-Lys-conjugate polyrotaxane significantly impeded the cellular uptake of a Gly-Sar dipeptide, a known human peptide transporter substrate, without being absorbed themselves. Notably, unconjugated polyrotaxanes showed no inhibitory effect, while monovalent Val-Lys-modified α-CD exhibited only a slight inhibition. These findings strongly suggest that simultaneous multivalent binding to several human peptide transporter proteins is essential for achieving effective inhibition, highlighting the potential of this CD-based polyrotaxane approach in modulating peptide transport and potentially developing new therapeutic strategies.

Subsequently, the same group expanded their investigation into a new CD-based polyrotaxane inhibition system by exploring cationic ligands. They developed dimethylaminoethylcarbamoyl-modified polyrotaxanes (Fig. 5C), which demonstrated remarkable efficacy in inhibiting L-Carnitine uptake by the intestinal organic cationic transporter. The study revealed that inhibition potency increased with longer polyethylene glycol chains, likely due to their ability to interact with multiple organic cationic transporter receptors simultaneously. Intriguingly, the inhibitory effect remained largely consistent regardless of the number of cationic ligands when combined with longer polyethylene glycol chains. Notably, these polyrotaxanes exhibited low cytotoxicity at therapeutically relevant doses, enhancing their potential for clinical applications. These observations support the hypothesis that the molecular mobility of ligand-conjugated α-CD plays an important in the multivalent binding capability of polyrotaxanes. The versatility of this approach has led to the development of various polyrotaxane capable of multivalent binding interactions with diverse biological targets, including proteins, enzymes, and lipid membranes. Recent advancements by Yui et al. have further demonstrated the potential of this technology, showing that mannose- and carboxyl-modified α-CD can enhance polyrotaxane uptake by macrophages through selective binding to scavenger receptor proteins, opening new avenues for targeted drug delivery and therapeutic interventions[78,79].

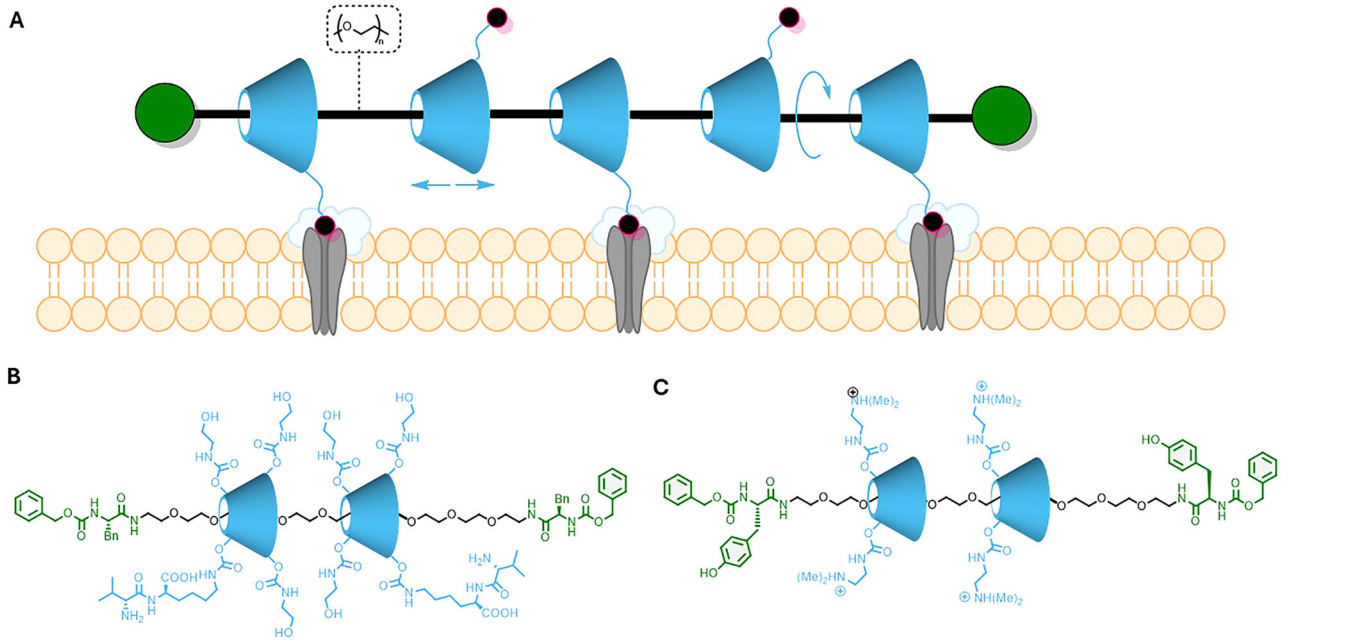

Fig. 5 | Structural design and multivalent protein binding of functionalized polyrotaxanes. A An illustration of the effect of "mobile" motion of the cyclic compounds in polyrotaxane on binding receptor proteins in a multivalent manner. **B** The structure of Val-Lys dipeptide polyrotaxane. **C** The structure of cationic dimethylaminoethylcarbamoyl polyrotaxane.

CD-based rotaxanes as transporters

Intracellular transportation of biomacromolecules such as DNA is pivotal for various therapeutic treatments. However, the size and the hydrophilic nature of most biomacromolecules pose significant challenges for transportation across the semi-permeable cell membrane[80]. Cationic biodegradable polyrotaxanes that can effectively mask the negative charge of biomacromolecules can be a useful tool to address these challenges[81,82]. Indeed, in 2006 Yui et al. have reported a modification of their α-CD-based cationic polyrotaxane as a promising transporter of a non-viral plasmid DNA[83]. This biodegradable cationic polyrotaxane formed stable polyplexes with plasmid DNA at significantly lower N/P ratios compared to linear polyethyleneimine or free dimethylaminoethylcarbamoyl-α-CDs, highlighting the importance of CD mobility in the polyrotaxane structure to facilitate tight polyplex packing. The efficacy of transfection using these polyrotaxane-based systems was found to be highly dependent on the number of α-CDs and the total number of dimethylaminoethylcarbamoyl groups. An optimal balance was required to form stable polyplexes that could effectively protect the plasmid DNA during cellular uptake while still allowing for timely intracellular release. Polyrotaxanes with intermediate numbers of α-CDs and amino groups demonstrated the best transfection ability, emphasizing the importance of optimizing DNA release timing for maximum transcription efficiency. Furthermore, biodegradable cationic polyrotaxanes have proven effective in transporting small interfering RNA (siRNA) intracellularly, despite the challenges posed by siRNA's short base-pair lengths and rigid secondary structure, which weaken electrostatic interactions and destabilize polyplexes. To enhance intracellular uptake and gene silencing activity, researchers carefully adjusted the number of threaded α-CDs and cationic groups. High numbers of α-CD threads were found to stabilize siRNA polyplexes significantly, boosting intracellular uptake by up to 27 times compared to linear poly(ethyleneimine). Initially, polyplexes struggled with endosomal escape due to low concentrations of glutathione, which limited disulfide cleavage. This challenge was addressed by replacing disulfide linkages with 3-sulfanylpropionyl ester linkages that are sensitive to the acidic pH of endosomes. Cleavage of these linkers triggered polyplex dissociation and destabilized the endosomal membrane by removing phospholipids and cholesterol, facilitating siRNA escape into the cytosol. In addition to siRNA, cationic polyrotaxanes have successfully delivered and reactivated anionic enzymes like β-galactosidase in living cells. This CD-based polyrotaxane approach to transport biomacromolecule offers a promising solution to the challenges of intracellular transport, combining efficient cellular uptake with controlled release mechanisms. The versatility and tunability of polyrotaxane systems make them attractive candidates for a wide range of therapeutic and research applications involving the delivery of large, hydrophilic biomolecules.

CD rotaxanes for medical imaging

Magnetic resonance imaging (MRI) is an advanced imaging method known for its high resolution and non-invasive nature, crucial in modern diagnostic medicine. To enhance MRI images, contrast agents like Gd^{3+} chelates are often used. However, most of the clinically approved Gd^{3+} chelates provide only moderate contrast improvement and rapid renal clearance, which affects contrast to surrounding tissues. To address these limitations, Zhou et al. introduced a novel approach using a CD-polyrotaxane scaffold[84,85]. In their pioneering work, they synthesized a family of highly water-soluble CD-polyrotaxanes using different Pluronic polymers. The rotaxanes were attached to Gd^{3+}-DO3A MRI contrast via β-CD rings and have cholesterol stoppers (Fig. 6A). This modification resulted in a threefold increase in ionic relaxivity compared to clinical agents like Gd^{3+}-DO3A. Importantly, the polyrotaxane structure also prolonged the circulation time in the bloodstream, allowing for more detailed anatomical imaging (Fig. 6B).

The improved relaxivity of the polyrotaxane structure was attributed to the mechanical bonding that restricted the rotation of the β-CD units. This constraint facilitated closer proximity and hydrogen bonding between the Gd^{3+}-DO3A chelates, thereby decreasing their molecular rotational correlation time—a critical factor influencing contrast agent efficiency. Subsequent studies demonstrated that polyrotaxane structures with multiple threaded Gd^{3+}-chelate functionalized β-CD units not only enhanced relaxivity further but also exhibited extended blood circulation times[85]. This extended circulation was likely due to increased binding to plasma proteins, facilitated by the higher molecular weight of these polyrotaxanes. Concisely, the development of CD-based polyrotaxanes as an MRI contrast agent represents a significant advancement in enhancing imaging quality while

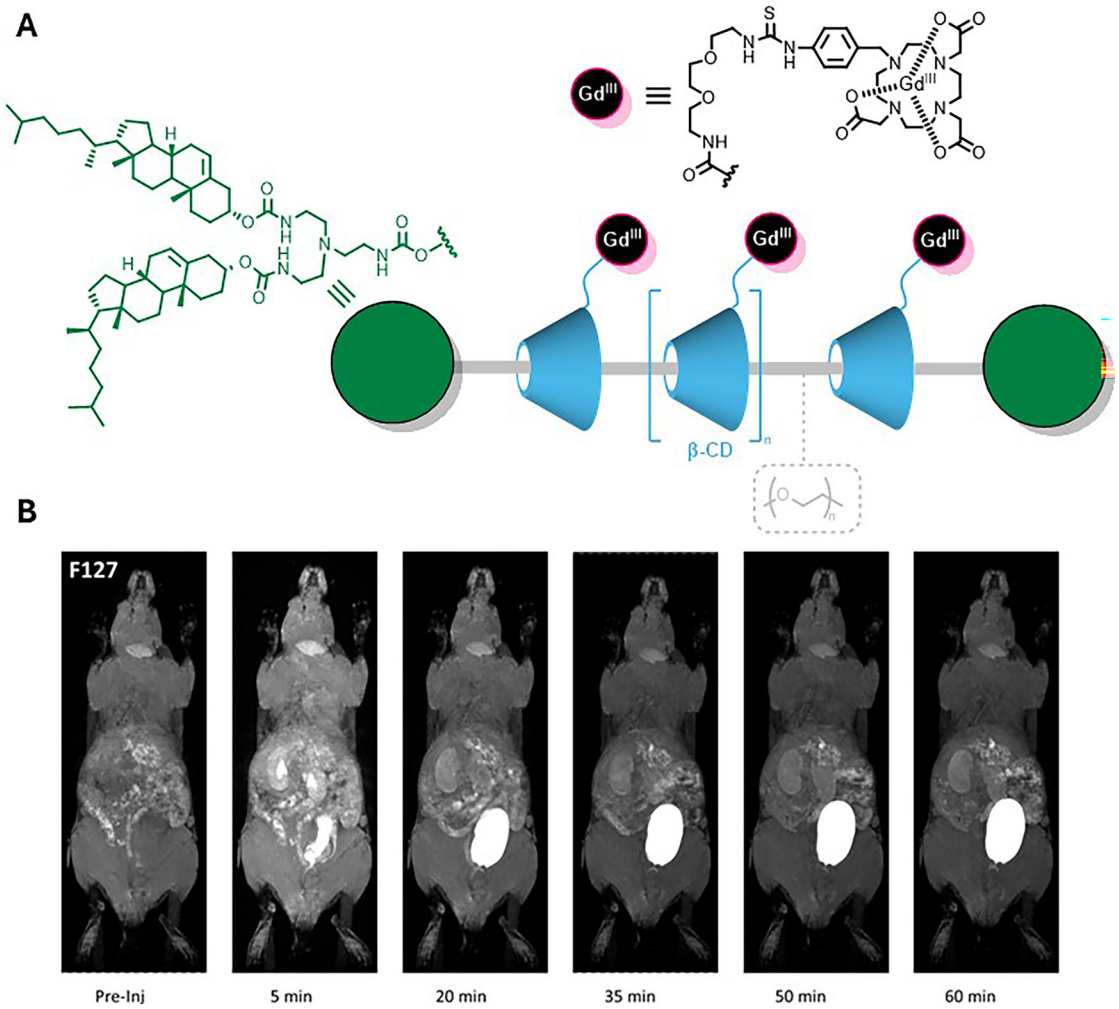

Fig. 6 | Structure and in vivo MRI performance of a Gd^{3+}-DO3A-modified β-CD-based polyrotaxane. A Structure of the Gd^{3+}-DO3A-modified β-CD-based polyrotaxane contrast agent. **B** MRI images show contrast agent distribution in mice up to 60 min after tail vein injection.

overcoming the limitations of conventional Gd^{3+} chelates. These results underscore the potential of CD-based polyrotaxanes in improving diagnostic precision and efficacy in clinical trials. Arguably, while complex polyrotaxanes offer high relaxivity, simpler [2] or [3]-rotaxanes may be advantageous for in vivo studies where rapid renal clearance is desired, as demonstrated by Fredy et al.[86]. In this study, Fredy et al. demonstrated that CD-based [3]-rotaxanes functionalized with Gd^{3+}-DO3A (attached to α-CD) exhibited enhanced kidney retention and contrast compared to gadoteric acid, likely due to its smaller size and increased hydrophilicity. In this case, in vivo studies could be undertaken in mice, with enhanced contrast and retention in the kidneys compared to a Food and Drug Administration-approved gadoteric acid[86]. It should be pointed out that the immense success of CD-based polyrotaxane as an MRI contrast agent was, from our perspective, the main impetus for the design of new systems based on CD-rotaxanes to be used as dual-modality agents[87].

The use of dual modality imaging agents has become a fascinating and booming area in medicinal chemistry. Generally, this approach integrate two imaging modalities in a single molecular scaffold[88,89], and perhaps one of the simple and persuasive term highlighting the demand of these agents is "two is better than one" reported by Long et al.[90]. Rotaxanes in particular can be an ideal and an attractive molecular scaffold for this end. Indeed, Hasenknopf et al. have reported a polyrotaxane system based on functionalized CDs with BODIPY fluorescent tag or Gd^{3+} complex as dual-modality imaging agents[87]. In this pioneering work, the authors show that the fluorescent properties of the BODIPY tag remain unaltered within the polyrotaxane structure, making it valuable for fluorescence imaging applications. The Gd^{3+} complex exhibits relaxivity approximately five-fold greater than the commercial Gadoteric acid. This significant enhancement in relaxivity, combined with the preserved fluorescence, renders these polyrotaxanes promising for use as dual modality imaging agents using both optical and MRI techniques. More recently, Holland et al. has reported a novel approach using combination of cucurbit[6]uril and CD-based macrocycles to develop a dual modality imaging agents for cancer biomarker imaging[91–93]. In this study, Holland and co-workers demonstrate the synthesis of metallo[4]rotaxanes incorporating radioisotopes (^{68}Ga or ^{89}Zr) and fluorescent probes, utilizing β-CD and cucurbit[6]uril-catalyzed cooperative capture synthesis[94]. The CD macrocycle plays a crucial role in host–guest chemistry, enabling the construction of these [4]rotaxanes that appeared to be stable and could be tuned to accommodate different radiometal ion complexes, proteins/peptides, and fluorophores for optical imaging. In addition, due to the relative ease of chemically modifying CDs, the authors have conjugated a cancer-targeted ligand to the CD and used it for the synthesis of targeted rotaxanes that showed high tumor uptake and specificity for HER2/neu-expressing xenografts in mice when radiolabeled with ^{89}Zr and conjugated to the monoclonal antibody Trastuzumab. A follow-up study showed that the expansion of this technology to synthesize ^{8}Ga-radiolabelled asymmetric rotaxanes via 4/6-components allowed evaluation of cellular uptake and binding of the radiolabeled rotaxanes in PSMA-positive and PSMA-negative cell lines[92]. The experimental results underscored the feasibility of using CD and cucurbit[6]uril-based

asymmetrical rotaxane platforms to develop dual-modality imaging agents that specifically target prostate cancer cells. This approach represents a promising advancement in molecular imaging for cancer diagnostics.

From the above, it should be obvious that the use of CD-based MIMs in medical imaging took off rapidly because of the promising in vitro and in vivo results of these molecules. This crucial field was poised to adopt the use of CD macrocycles because of their biocompatible properties. This, combined with the flexibility of CDs for chemical modifications and their usefulness to make interlocked structures, has allowed CD-based MIMs to become the starting point for many researchers in this field.

CD rotaxanes for biomaterial applications

Due to their biocompatibility and highly tunable three-dimensional aqueous networks, CD-rotaxanes have also proven to be promising candidates as novel biomaterials for use in regenerative medicine[95], self-healing materials[96], and hydrogels[97]. For example, polyethylene glycol-α-CD-polyrotaxane hydrogels have been used for the encapsulation and controlled intracellular delivery of poorly water-soluble compounds[98]. By altering the number of α-CD units and degree of crosslinking, the rate of hydrogel degradation through hydrolytic ester cleavage could also be controlled to slowly release 6-aminofluoroscein in vitro over 2–8 days. Recent studies report polyrotaxane hydrogels as efficient, biocompatible crosslinkers for collagen, achieving up to 87.1% crosslinking via imine formation —far higher than conventional methods[99]. Polyrotaxane-crosslinked collagen shows improved tensile strength, thermal and enzymatic stability, and negligible cytotoxicity[100]. In these hydrogels, the use of CD-polyrotaxanes is mainly to overcome the structural inhomogeneity caused by the traditional covalent crosslink, which often leads to compromised mechanical strength and potential cytotoxicity[101]. We refer the readers to more comprehensive sources for obtaining information on the use of CD-rotaxanes for biomaterials applications[36,102,103].

Challenges and limitations

Despite the promise of CD-rotaxanes in biomedicine, significant challenges impede their journey from bench to real-world application. Scale-up synthesis is a major hurdle. Current synthetic routes often rely on multi-step procedures and template-directed strategies involving stoichiometric amounts of bulky end-capping agents. These methods are inherently inefficient and difficult to adapt to large-scale production. While continuous flow reactors offer a potential solution, the development of robust, high-yielding, and cost-effective synthetic protocols remains a critical need. In addition, the aqueous environment requirements for native CD threading limit reactions, though permethylation[25], co-solvents[104], and ionic liquids[105] offer workarounds; however, most CD-rotaxane syntheses still use aqueous systems due to challenges in achieving high yields and functional group tolerance in non-polar media. Furthermore, regulatory approval pathways for MIMs are not yet well-defined. As MIMs are not explicitly addressed by existing pharmaceutical guidelines, clear frameworks for safety assessment and clinical evaluation are needed. Close collaboration between researchers, regulatory agencies, and industry partners is essential to define appropriate standards and facilitate the translation of CD-rotaxane-based therapeutics. In vivo ADME (absorption, distribution, metabolism, excretion) properties require further investigation; preliminary studies suggest that CD-rotaxane clearance is highly dependent on size, charge, and degree of functionalization. Immunogenicity is also a concern, particularly for larger polyrotaxanes. Production costs are also considered a major limit; today, the cost of CD-rotaxane synthesis limits their widespread adoption in clinical settings.

Outlook

As we discussed above, CD-based rotaxanes have emerged as versatile supramolecular architectures with significant potential in biological and medicinal applications. Throughout this review, we have explored their diverse uses, including drug targeting and delivery, medical imaging, controlled drug release, enzyme inhibition, biomolecule transport, and as theranostic agents. The majority of these applications have predominantly utilized polyrotaxanes, which have demonstrated remarkable utility across various biomedical fields. While polyrotaxanes have been extensively studied and have shown promise, our perspective is that simpler CD-based rotaxanes, particularly [2]-rotaxanes, hold even greater potential for clinical applications. These simpler structures offer several advantages, including enhanced pharmacokinetics, more precise structural control, and easier synthesis and characterization. Smaller rotaxanes may provide improved biodistribution and clearance profiles, potentially reducing off-target effects and allowing for more defined structures that enable better control over drug release and targeting[17]. These perspectives can be drawn from similar studies done for several MIMs with other synthetic macrocycles[106].

To fully realize the potential of simple CD-based rotaxanes, future research efforts should be devoted to developing efficient template-directed synthesis methods[107–110]. These methods should aim to control the formation of rotaxanes with high yield and purity. Additionally, exploring novel end-capping strategies to create stable rotaxanes while maintaining their responsiveness to biological stimuli will be crucial. Alternatively, development of stimuli responsive CDs could be a powerful tool to be used in biologically related studies[111–114]. Investigating the impact of different CD sizes (α, β, γ) on the properties and applications of rotaxanes could also yield valuable insights. By focusing on these areas, researchers can unlock the full potential of CD-based rotaxanes, paving the way for a new generation of precisely engineered supramolecular systems in biological and medicinal applications.

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S.P., H.A., and A.S. planned the manuscript, prepared the text, and commented on final drafts.

Competing interests

The authors declare no competing interests.

Additional information

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