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Enzyme-responsive supramolecular polymers by complexation of bis(*p*-sulfonatocalixarenes) with suberyl dicholine-based pseudorotaxane†

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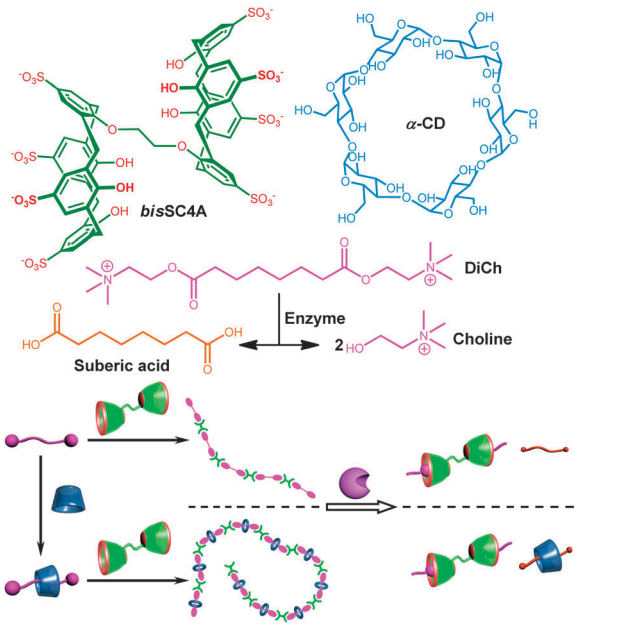
A linear supramolecular ternary polymer was fabricated by iteratively threading cyclodextrin with suberyl dicholine and end-capping with bis-calixarenes, showing desired cholinesterase response.

Supramolecular polymers, emerging from the combination of supramolecular chemistry and polymer science, are polymers based on monomeric units held together by directional and reversible non-covalent interactions.[1] Compared with covalent polymerization, the non-covalent route is emerging as a smart design principle for responsive materials capable of self-healing and delivery.[2] To achieve supramolecular polymerization, the strength of the intermolecular binding is foremost, since high complexation stabilities are always demanded to build truly polymeric materials.[3] On the other hand, spacers between two (self-) complementary recognition sites also play a crucial role in directing supramolecular polymerization, including length and flexibility factors.[4] However, such desirable spacers are not always handy to build supramolecular polymers from the viewpoints of tedious synthesis and other shortcomings that would be encountered. Inspired by the dynamic nature of supramolecular polymers, regulating spacers by a non-covalent approach has attracted more and more interest recently.

Rotaxanes are mechanically-interlocked supramolecular architectures consisting of a dumbbell shaped molecule which is threaded through a macrocycle, which has been widely applied in molecular machines and devices.[5] Integration of rotaxanes with supramolecular polymers will form novel assembly species with fascinating properties.[6] The wheel of rotaxane plays a crucial role in generating the well-defined architectures. Very recently, we constructed a viologen@cucurbituril–calixarene ternary polymer, showing that the cucurbituril wheel can effectively modulate the topology of the supramolecular assemblies.[7]



Scheme 1 Structural illustration of the building blocks and schematic representation of the resulting enzyme-responsive supramolecular polymers.

As part of our ongoing program concerning supramolecular polymers based on macrocycles, we report here an enzyme-responsive supramolecular polymer with suberyl dicholine (DiCh) as the axle, α-cyclodextrin (α-CD) as the threading wheel, and bis(*p*-sulfonatocalix[4]arenes) (bisSC4A) as the iterative end-capping unit (Scheme 1). On account of the hydrolysis of DiCh by cholinesterases, the present assembly is capable of dispersal by an enzymatic reaction. Among a variety of stimuli employed to manipulate responsive assemblies, the enzyme approach exhibits numerous advantages such as inherent biocompatibility, high efficiency and specificity, and mild conditions.[8]

DiCh was prepared as the homoditopic candidate guest for calixarene, since it is well-known that *p*-sulfonatocalix[4]arene (SC4A) shows high binding affinity to choline species,[9] and moreover, the spacer formed by esterification of suberate with two cholines is long enough to span the cavities of two SC4A

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hosts.[4f] Upon addition of bisSC4A, the terminating choline protons of DiCh undergo pronounced upfield shifts, whereas the suberate spacer protons display almost negligible shifts (Fig. S3, ESI†). It indicates that DiCh is captured by bisSC4A with quaternary ammonium moieties deeply immersed into the cavity, but the suberate spacer is located outside. The binding constant was obtained as $(1.2 \pm 0.2) \times 10^4$ M^{-1} (Fig. S4, ESI†). It is reasonably acceptable that the iterative complexation between bisSC4A and DiCh would lead to the formation of a higher order supramolecular polymer or cyclic species.

A simple 1 : 1 mixture of bisSC4A and DiCh (1.0 mM) in aqueous solution exhibits pronounced scattering intensity. Dynamic light scattering (DLS) results show a broad size distribution with an average diameter of 337 nm and a scattering intensity of 95 kcps (Fig. 3 and Fig. S9a, ESI†). Diffusion-ordered spectroscopy (DOSY) experiments further validated the formation of a large-sized assembly,[10] displaying the diffusion rate (1.72×10^{-10} m^2 s^{-1} at 20 mM) for bisSC4A and DiCh in an equimolar mixture (Fig. S12, ESI†). That is, both complexed bisSC4A and DiCh diffuse together as one entity with a diffusion rate definitely slower than that of free DiCh (4.32×10^{-10} m^2 s^{-1} at 20 mM) and bisSC4A (2.01×10^{-10} m^2 s^{-1} at 20 mM). More significantly, the diffusion rate of DiCh@bisSC4A is strongly dependent on its concentration. Upon concentrating the solution from 1 to 40 mM, the diffusion rate decreases from 2.00×10^{-10} to 1.46×10^{-10} m^2 s^{-1} (Fig. 1). The extremely differential diffusion rates as a function of concentration indicate considerably concentration-dependent hydrodynamic radii, displaying the formation of the supramolecular polymer. The average polymerization number was estimated to be 22 at 20 mM according to the binding constant.[6j] The morphology of the supramolecular binary polymer formed by the complexation of bisSC4A with DiCh was confirmed by TEM images (Fig. 2a), showing the 1D linear structure.

In terms of ternary assembly, it is a prerequisite to prepare the pseudorotaxane by threading α-CD with DiCh. The 2D ROESY spectrum exhibits clear cross-peaks between the α-CD protons and the spacer protons of DiCh (Fig. S5, ESI†), which proves the formation of pseudorotaxane DiCh@α-CD. The binding constant of α-CD with the alkyl cation is in the order of 10^3 M^{-1},[11] and thereby, the threading should be effective at the operating concentration. Further addition of bisSC4A to DiCh@α-CD leads to similar upfield shifts of the quaternary ammonium protons to binary DiCh@SC4A, indicating that the formation of pseudorotaxane does not interfere with the complexation of calixarene with choline (Fig. S7, ESI†).

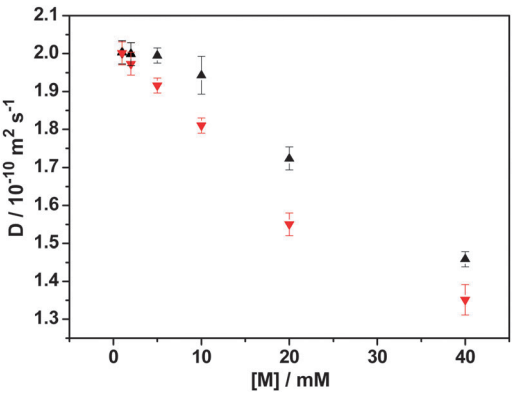

Fig. 1 Concentration dependence of the diffusion coefficient of DiCh@bisSC4A (black) and DiCh@α-CD–bisSC4A (red).

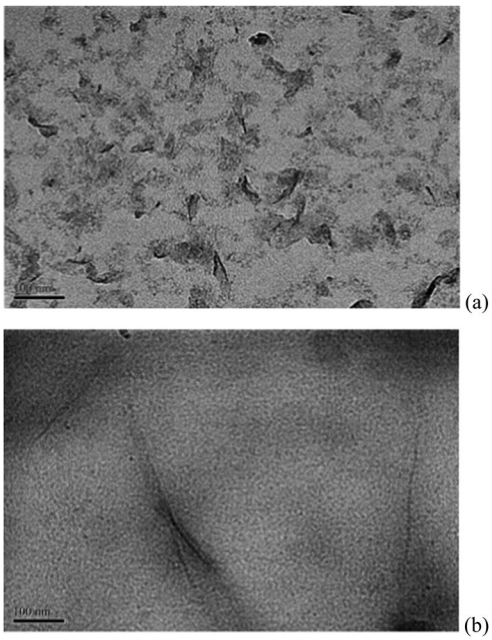

Fig. 2 TEM images of the binary DiCh@bisSC4A (a) and the ternary DiCh@α-CD–bisSC4A (b) (scale bar = 100 nm).

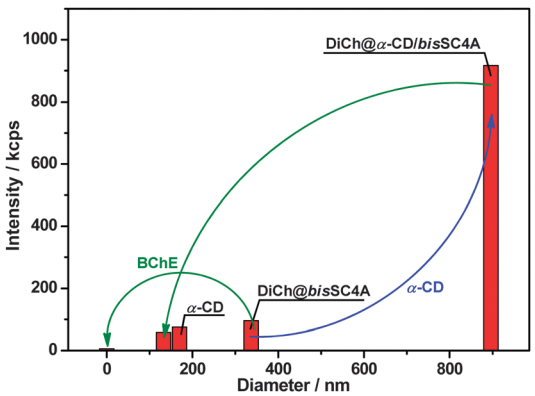

Fig. 3 Averaged diameters and scattering intensities of DiCh@bisSC4A ([DiCh] = [bisSC4A] = 1.0 mM) and DiCh@α-CD–bisSC4A ([DiCh] = [bisSC4A] = 1.0 mM, [α-CD] = 10 mM) in the absence and presence of BChE (10 U mL^{-1}, 24 h of incubation). The average diameter and scattering intensity of 10 mM α-CD was also examined as a control experiment.

Compared with DiCh@bisSC4A, DiCh@α-CD–bisSC4A exhibits not only a larger hydrodynamic diameter (896 nm), but also much stronger scattering intensity (917 kcps) (Fig. 3 and Fig. S9b, ESI†). The DLS results demonstrate the stimulative effect of pseudorotaxane on supramolecular polymerization. DOSY experiments further confirmed the formation of a ternary DiCh@α-CD–bisSC4A polymer; the diffusion coefficient decreases nonlinearly from 2.00×10^{-10} to 1.35×10^{-10} m^2 s^{-1} upon concentrating the solution from 1 to 40 mM (Fig. 1). Moreover, the three components diffuse together as one entity with a diffusion rate (1.55×10^{-10} m^2 s^{-1} at 20 mM, Fig. S12, ESI†) considerably slower than that of binary DiCh@bisSC4A. The improvement of

supramolecular polymerization can also be observed from the TEM images (Fig. 2b). Under the same concentration and conditions, DiCh@bisSC4A presents a short linear morphology of about 50 nm, whereas DiCh@α-CD–bisSC4A presents a long one of about 250 nm. Regulation of supramolecular polymerization was therefore successfully addressed by simple threading instead of tedious covalent syntheses.

DiCh is a homoditopic alkanoylcholine that can be hydrolyzed to suberic acid and choline by cholinesterases.[12] It is therefore anticipated that the present assemblies could be dispersed by the action of enzymes. Upon addition of butyrylcholinesterase (BChE), both DiCh@bisSC4A and DiCh@α-CD–bisSC4A were degraded, reflecting not only the decreased size distribution but also the weakened scattering intensity (Fig. S10, ESI†). As shown in Fig. 3, the DiCh@bisSC4A assembly is completely dispersed upon incubation with BChE. In the case of DiCh@α-CD–bisSC4A, the average diameter decreased from 896 to 132 nm, and the scattering intensity decreased from 917 to 58 kcps. It seems that BChE did not degrade the ternary polymer thoroughly. A close examination shows that 10 mM free α-CD gave a DLS signal too (Fig. S11, ESI†), with an average diameter of 170 nm and a scattering intensity of 75 kcps. This is because free α-CD forms aggregates at higher concentrations.[13] Comparing the DLS signals of DiCh@α-CD–bisSC4A after incubation with BChE and free α-CD, we inferred that the ternary polymer has been completely dispersed by BChE. The process of enzymatic reaction was also monitored using ESI-MS spectroscopy (Fig. S14, ESI†); the molecular ion peak of DiCh disappeared, and only that of choline was observed. After enzymatic hydrolysis of DiCh, the choline product was captured by bisSC4A and suberic acid was captured by α-CD (Fig. S6 and S8, ESI†).

In summary, we have successfully fabricated a novel supramolecular ternary polymer DiCh@α-CD–bisSC4A together with a binary polymer DiCh@bisSC4A. Compared with binary DiCh@bisSC4A, ternary DiCh@α-CD–bisSC4A exhibits not only a larger polymeric size but also better size distribution and topology, which result from the integration of pseudorotaxane with the supramolecular polymer. Threading α-CD on DiCh effectively regulates the spacer flexibility, and therefore boosts the supramolecular polymerization. Although DiCh is concurrently captured by both α-CD and bisSC4A, the obtained assemblies are still enzyme-responsive on account of the dynamic equilibrium characteristics of non-covalent interactions. As an appealing perspective, the present study certainly represents a proof of principle that these systems could be applied as biodegradable materials.

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