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CO_2-Switchable Supramolecular Block Glycopolypeptide Assemblies

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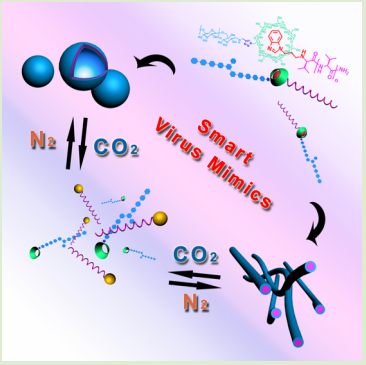
Supporting Information

ABSTRACT: A novel supramolecular block glycopolypeptide, designed to have the viral building blocks and be sensitive to CO_2, a physiological stimulus, was prepared via the orthogonal coupling of two end-functionalized biopolymers, dextran with β-cyclodextrin terminal (Dex-CD) and poly(L-valine) with a benzimidazole tail (BzI-PVal), respectively, driven by the end-to-end host–guest interactions. Due to the CO_2-cleavable CD/BzI connection, both the vesicular and fibrous aggregates of this supramolecular block copolymer self-assembled in aqueous solution can undergo a reversible process of disassembly upon "breathing in" CO_2 and assembly upon "breathing out" CO_2, which mimics, to some extent, the disintegration and construction of viral capsid nanostructures.



Supramolecular block copolymers, defined as a class of dynamic macromolecular adducts, have developed bloomingly in recent years owing to their particular applications in biomaterials and drug delivery.[1−3] They can be interconnected by two or more homopolymers via chain-terminal noncovalent interactions including H-bonding and metal–ligand interaction.[4−12] Because of the dynamic nature and the reversibility of these connections, they offer infinite possibilities to predesign the polymeric properties and functions. Some nascent works have been devoted to preparing smart aliphatic chain-type supramolecular copolymers through introducing a stimuli-responsive noncovalent junction.[13−19] For example, the Zimmerman group reported a supramolecular diblock copolymer in which the quadruple H-bonding connector can reversibly associate and dissociate responding to a redox agent,[13] and Yuan et al. exploited a kind of host–guest pseudocopolymer, which can be fractured by UV light irradiation.[14] Since these exogenous stimuli have side effects to biological cells including chemical regent contamination, cytotoxicity, and gene damage, developing new trigger modes adapted to intracellular application is an emerging challenge. In this regard, carbon dioxide (CO_2), as a key endogenous cell metabolite with good biocompatibility, is a promising candidate to overcome these issues. Despite recent progress on CO_2-sensitive covalent block copolymers,[20−22] using CO_2 as a physiological trigger to control supramolecular block copolymer assemblies has not been reported thus far.

Viral capsid, as an essential structure of a virus, is composed of a peptide inwall and a saccharide coat, which can further self-organize into different viral nanoparticles such as fibers and vesicles. In a typical cell invasion process, the viral inner peptide can be dissociated from the outer glycan blocks by specific biostimulation. Biomimicking the viral structures and their stimuli-responsive behaviors is of great interest and represents one step toward the monumental challenge of synthesizing natural viruses.[23−26]

Herein, we have developed a class of supramolecular block glycopolypeptides to simulate the component of natural viral capsids and their controllable self-assembly and disassembly process by CO_2 trigger. As shown in Scheme 1, the noncovalent glycopolypeptides are orthogonally coupled by two end-functionalized biopolymers, dextran with β-cyclodextrin distal (Dex-CD) for mimicking the outer saccharide coat of the viral capsid and poly(L-valine) containing a benzimidazole terminal (BzI-PVal) for the inner peptide layer, through the CD/BzI host–guest interactions. Normally, the uncharged BzI species is bound in the hydrophobic cavity of CD to form a 1:1 inclusion complex; however, when CO_2 is added in the solution of this complex, BzI species can be protonated and convert into the charged BzI^+ form, which is excluded out of the host rapidly.[27] Since this process can be reversibly switched by CO_2, we expected that our supramolecular block glycopolypeptides can further self-assemble into a variety of multidimensional nanoobjects. These virus mimics can respond to the CO_2 gas for undergoing a self-assembly and disassembly process by means of reversible linkage and cleavage of the Dex-CD/BzI-PVal host–guest adducts.

Typically, the macromolecular host, Dex-CD, was prepared by click chemistry between 6-monoazido-β-CD and alkynyl-dextran, and the guest, BzI-PVal, was obtained by L-valine *N*-carboxyanhyride ring-opening polymerization (synthetic details in Supporting Information). Preliminary studies were carried out to prove whether the two biopolymers can orthogonally form a supramolecular block glycopolypeptide. Injecting an

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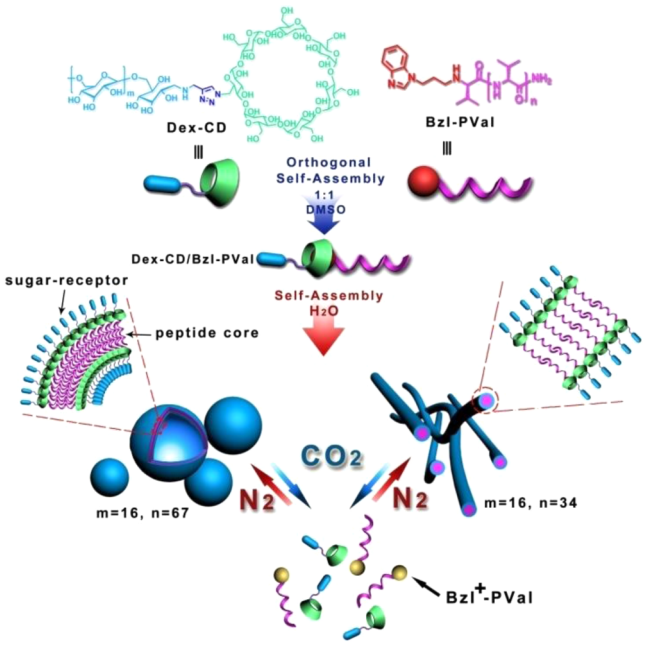
Scheme 1. Orthogonal Connection of End-Decorated Polysaccharide (Dex-CD) and Poly(L-valine) (BzI-PVal) to Form Supramolecular Block Glycopolypeptides and Schematic of Their CO_2-Switchable Assembly and Disassembly Behavior Mimicking the Diversiform Viral Capsids



equal amount of Dex-CD in dimethyl sulfoxide (DMSO, 0.1 mM, 5.0 mL) into the BzI-PVal DMSO solution enables an end-to-end complexation, as suggested by the formation of a translucent colloidal (Figure S3, Supporting Information). Gel permeation chromatography (GPC) experiments further ascertained the host–guest pseudocopolymer and its CO_2-sensitive dissociation (Figure 1): single Dex-CD or BzI-PVal

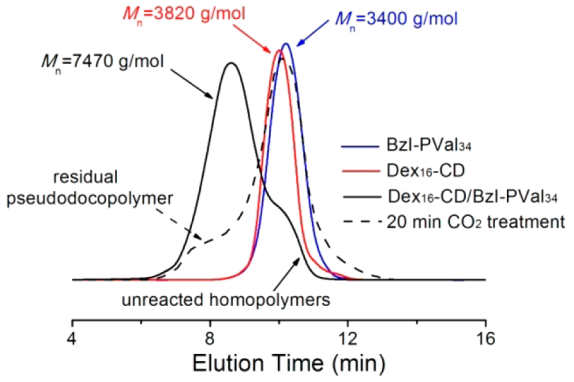


Figure 1. GPC traces showing the orthogonal coupling of BzI-$PVal_{34}$ (blue line) and Dex_{16}-CD (red line) into the supramolecular host–guest adducts (Dex_{16}-CD/BzI-$PVal_{34}$ in 1:1 stoichiometry, black line) and the subsequent CO_2-sensitive dissociation of the pseudocopolymer (20 min gas stimulation, black dash line).

showed high elution time (~10 min) due to its smaller molecular weight (blue and red line). Mixing the two moieties in DMSO for 2 days, a shoulder peak of GPC trace appeared. The main peak shifted to lower elution time from 10 down to 8.6 min, indicating a dramatic increase of molecular weight from 3.4 to 7.5 kDa (black line). It accords with the change from a smaller homopolymer to a larger macromolecular adduct. Another shoulder peak with weaker strength (9.8–10.5 min) is consistent with the unreacted homopolymers. From the integral area of the main and shoulder peaks, the efficiency of Dex-CD and BzI-PVal complexation is calculated to be 86%. When CO_2 was bubbled into the binary system for 20 min, interestingly, with the solution pH decrease from 7.4 to 5.8, the GPC trace almost reverted back, which means that CO_2 can cut off these macromolecular adducts (black dash line). However, a shoulder peak at lower elution time remained, implying that this CO_2-driven cleavage is incomplete and the dissociation efficiency is 82%. These findings confirm that Dex-CD and BzI-PVal can noncovalently form saccharide–peptide via the CO_2-sensitive host–guest interaction.

After proving the formation of the pseudocopolymer, we wondered how strong the host–guest complexation was. To elucidate this question, isothermal titration calorimetry (ITC) was employed to directly measure the binding affinity.[28] In the experiment, Dex-CD and a water-soluble BzI-poly(ethylene oxide) (BzI-PEO) analogue were chosen as models. The BzI-PEO (2.0 mM) was dripped into the Dex-CD aqueous solution (0.1 mM). An exothermic isotherm yielded an association constant (K_t) of 2.65 × 10^4 M^{-1} (Figure 2a), which is

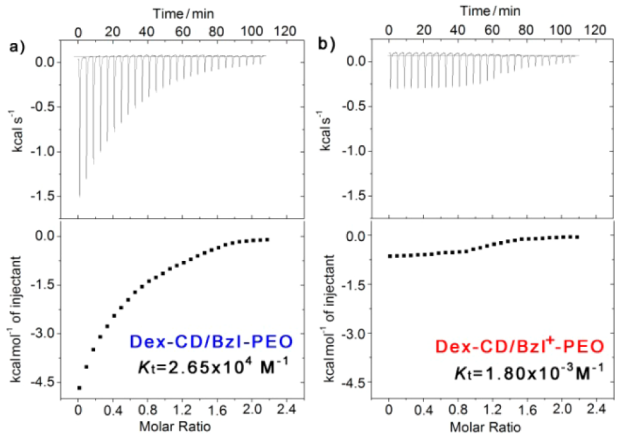


Figure 2. ITC data for the isothermal titration of Dex-CD (0.1 mM) with water-soluble BzI-PEO (2.0 mM) in water: (a) host–guest complexation in the absence of CO_2 stimulus, (b) host–guest decomplexation after exposure to CO_2 for 20 min. All the experiments were performed at 25 °C.

corresponds to the results from the Benesi–Hildebrand equation by UV–vis spectra (K_t = 2.2 × 10^4 M^{-1}; Figure S4, Supporting Information).[29,30] Upon CO_2 treatment for 20 min, the BzI-PEO is protonated and converted to a charged BzI^+-PEO moiety. In this case, the binding affinity significantly decreased to K_t = 1.80 × 10^{-3} M^{-1} (Figure 2b), indicating the CO_2-switchable dissociation between the CD and BzI terminal connection.

We next turned our focus into whether the supramolecular block glycopolypeptide can self-assemble into virus-like nanoparticles. By tuning the peptide block length, two different pseudocopolymers, Dex_{16}-CD/BzI-$PVal_{67}$ and Dex_{16}-CD/BzI-$PVal_{34}$, were prepared. Dextran is a hydrophilic sugar receptor, whereas poly(L-valine) is known to adopt a hydrophobic β-sheet conformation by intermolecular H-bonding.[31,32] To elucidate their self-assembly in water, we used the nanoprecipitation technique.[33] An excess of deionized water was slowly added into the Dex-CD/BzI-PVal copolymer DMSO

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solution at a rate of 0.1 mL/h, and then after removal of DMSO by dialysis against water the dispersion was further characterized. The critical aggregate concentration was ~0.18 mg/mL for Dex_{16}-CD/BzI-$PVal_{67}$ and ~0.34 mg/mL for Dex_{16}-CD/BzI-$PVal_{34}$, respectively, as monitored by the fluorescent probe method (Figure S5, Supporting Information).[34] To visualize the size and morphological difference of the two assemblies, transmission electron microscopy (TEM) was employed. For the Dex_{16}-CD/BzI-$PVal_{67}$ with a longer peptide block, Figure 3a shows that the supramolecular glycopolypeptides can form

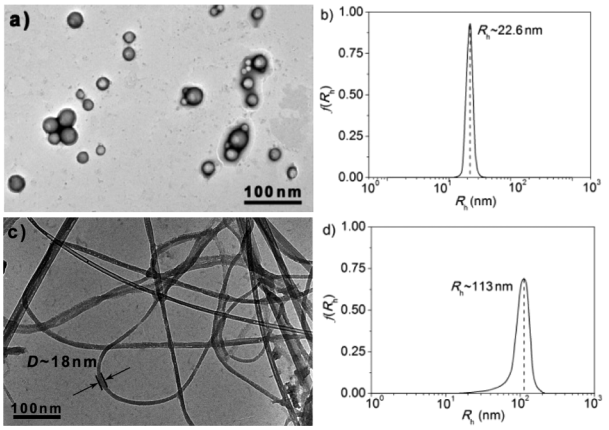

Figure 3. TEM images and DLS results for two types of supramolecular block glycopolypeptide assemblies: (a),(b) Dex_{16}-CD/BzI-$PVal_{67}$ for vesicle-like structures and (c),(d) Dex_{16}-CD/BzI-$PVal_{34}$ for fiber-like structures. The polymer sample concentration is at ~0.5 mg/mL.

small sphere-like aggregates. The clear contrast between the dark edge and the hollow center indicates that the spheres are vesicular. TEM images reveal their size of (32 ± 11) nm, corresponding to the hydrodynamic radius, R_h, of 22.6 nm determined by dynamic light scattering (DLS, Figure 3b). The deviation between TEM and DLS results ascribes to the fact that DLS takes into account the hydrated state while TEM, using freeze-dried samples, does not. The membrane thickness of these vesicles is 7–9 nm, suggesting an interdigitated membrane structure. For the sample of Dex_{16}-CD/BzI-$PVal_{34}$ with a shorter peptide segment, interestingly, they can self-assemble into a typical one-dimensional fibrous nanostructure with a large length/diameter ratio ($250 < L/D < 350$ statistics from 25 fibers): their diameter is on average 18 nm, indicating a bilayer molecular fashion, and their length exceeds several micrometers, which is consistent with the equivalent radius of 113 nm from DLS (Figure 3c,d). These supramolecular assemblies have similar composition and morphology with natural viral capsid nanoparticles. In addition, the geometrical difference of these two samples is governed by the hydrophilic volume fraction (f) of the block copolymer:[35] theoretically, spherical micelles should be formed when $f > 50\%$, worm-like micelles are favored as $40\% < f < 50\%$, and vesicular structures tend to form for $f < 40\%$. To our supramolecular copolymer samples, by increasing the length of the peptide block from 34 to 67 repeating units, the calculated f decreases from 48% to 37%, corresponding to the fibrous and vesicular shape, respectively. Importantly, it is worth noting that these supramolecular nanoparticles were stable over one month if no stimulus was applied.

As mentioned above, the viral capsid particles can undergo a dissociation effect in response to biological stimulation. We wanted to know whether these biomimetic viral assemblies possess CO_2-responsive controlled assembly and disassembly behavior. To test this point, we used TEM to track their morphological evolution. For the sample of small vacuolar-like virus mimics, after bubbling CO_2 for 10 min, the intact vesicles gradually disappeared, and the vesicular membrane began to crack and shatter as accompanied by the solution pH decrease from 7.8 to 6.9 (Figure 4a,b), which results from the CO_2-

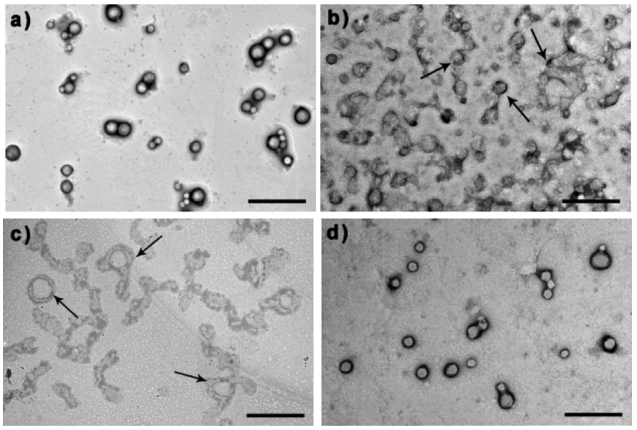

Figure 4. TEM images of the disassembly and reassembly of Dex_{16}-CD/BzI-$PVal_{67}$ vesicular virus mimics under different conditions: (a) no stimulus, (b) 10 min CO_2 stimulus, (c) 20 min CO_2 stimulus, and (d) 30 min N_2 stimulus (scale bar: 100 nm). The polymer sample concentration is at ~0.5 mg/mL.

sensitive dissociation of the supramolecular glycopolypeptide chains from the middle host–guest connection. Further purging with CO_2 gas to 20 min, the solution pH reached 5.7. In this case, the vesicular membrane architectures were totally destructed, and only a small number of membranous fragments were seen (Figure 4c, arrow), indicating a complete disassembly of these vesicle-like nanoparticles. Reversibly, upon nitrogen (N_2) bubbled into the solution for 30 min with ultrasound-prompted redissolution of those cleavable peptide chains, the noncovalent block saccharide-polypeptide can be reformed, resulting in the reassembly of the vesicles with similar shape and size (Figure 4d). Furthermore, UV–vis spectral analysis also supported the above results. In the absence of gas stimulation, the characteristic absorption at 264 nm of the assemblies is attributed to the CD/BzI host–guest complexes (Figure S6, Supporting Information, blue line), while upon CO_2 treatment, a hypochromatic shift from 264 to 252 nm and a large change of peak profile ascribed to the absorption of charged BzI^+ species both disclose that the vesicles have decomposed to Dex_{16}-CD and BzI^+-$PVal_{67}$ (Figure S6, Supporting Information, red dash line). By applying N_2 gas for removal of CO_2, the initial absorption can recover (Figure S6, Supporting Information, blue dash line), reflecting the rebuilding of Dex_{16}-CD/BzI-$PVal_{67}$ supramolecular vesicles.

For the one-dimensional fibrous system (Dex_{16}-CD/BzI-$PVal_{34}$), in a similar way, they also exhibited CO_2-switchable reversible degradation and reconstruction. After adding CO_2 to the solution for 10 min, these long fibers randomly produced some fracture points along their axes, leading to the shortening of the assemblies from micrometers down to tens or hundreds of nanometers (Figure 5a,b). Continuously prolonging the CO_2

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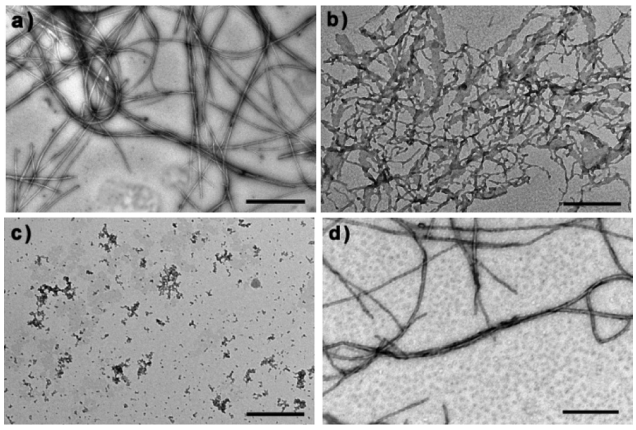

Figure 5. TEM images of the disassembly and reassembly of Dex_{16}-CD/BzI-$PVal_{34}$ fibrous virus mimics under different conditions: (a) no stimulus, (b) 10 min CO_2 stimulus, (c) 20 min CO_2 stimulus, and (d) 30 min N_2 stimulus (scale bar: 200 nm). The polymer sample concentration is ~0.5 mg/mL.

aeration time, all the fibers vanished with only irregularly shaped fragments remaining in solution (Figure 5c). It is possible that the cleaved BzI^+-PVal chains form small micellar aggregates due to their amphiphilicity with a charged BzI^+ headgroup and a short hydrophobic polypeptide tail. The observations indicate that Dex_{16}-CD/BzI-$PVal_{34}$ chains could delink by CO_2, and as a result, the assembling worm-like virus mimics are disassembled. The disintegration of the fibrous objects was also recorded by the count rate data from DLS. An abrupt counting reduction indicates the drop in the amount of the particles with the increase of CO_2 trigger time (Figure S7, Supporting Information). Like the case of vesicles, these one-dimensional nanostructures can be reverted back when applying N_2 (Figure 5d). Therefore, considering that these supramolecular glycopolypeptide assemblies biomimick the viral capsids from their structure and morphology, this CO_2-responsive self-disruption and self-construction of the virus-like nanoparticles is in many ways reminiscent of the biosignal-mediated natural viral disassembly.

In conclusion, we have developed a new class of CO_2-switchable supramolecular block glycopolypeptides. Formed by the orthogonal coupling of two end-decorated biopolymers, Dex-CD and BzI-PVal, the noncovalent polysaccharide–polypeptide copolymers can self-organize into high-ordered and multidimensional nanostructures to mimic the shape of natural viruses. The CO_2-cleavable CD/BzI host–guest block junction allows the biomimetic virus-like assemblies to display a reversible assembly and disassembly process tuned by CO_2. In view of the dynamic nature of the supramolecular linker and the mild gas trigger mode close to our physiological environment, we envisage that the supramolecular block glycopolypeptide assemblies might open up a new door to constructing smart virus-like particles for bioapplications.

■ ASSOCIATED CONTENT

Supporting Information

Details of synthesis, turbidity tests, CAC measurement, UV–vis spectra, and DLS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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