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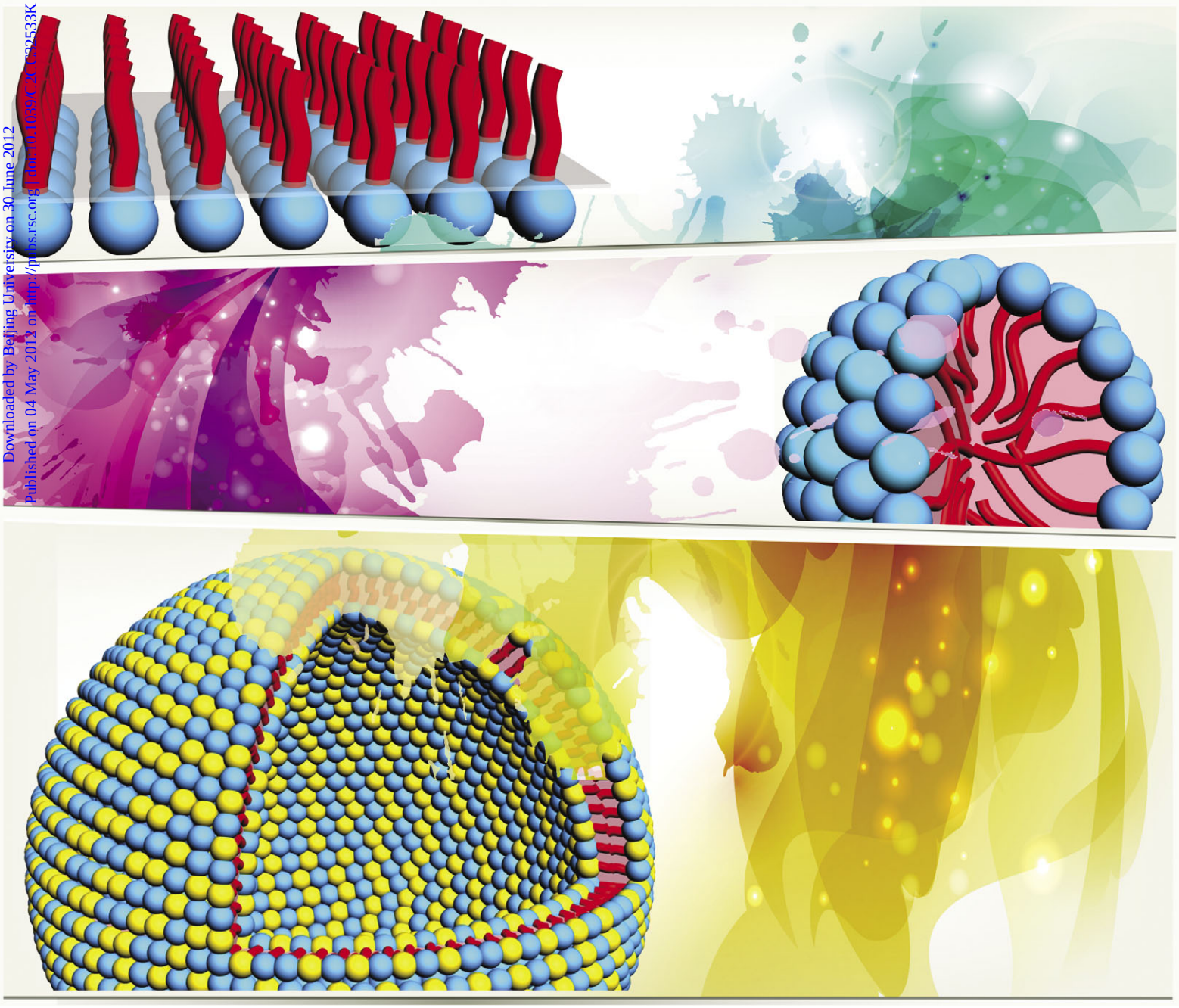

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Enzyme-triggered model self-assembly in surfactant–cyclodextrin systems†

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We present here a host–guest approach to construct enzyme-triggered assembly systems on the basis of surfactant–cyclodextrin complexes and α-amylase. We realized enzyme-responsive model self-assembly systems including monolayers, micelles, and vesicles. The host–guest approach is expected to be extended to more complicated assembly systems with widespread applications.

Molecular self-assembly, the spontaneous association of molecules into structurally well-defined entities, is ubiquitous throughout basic science, technology, and nature.[1] Over time, biological systems have developed many comprehensive self-assembly systems where stimuli-responsiveness is extensively involved to perform a function. Mimicking these stimuli-responsive systems is of increasing interest thanks to its wide applications from targeted drug delivery, sensors, to molecular diagnostics.[2] Among different stimuli, enzymes are an attractive and unique kind, because they are substrate specific, can amplify a response *via* catalytic reactions, and may have disease-associated expression patterns.[3,4] Usually, synthetic approaches are employed to develop "smart" molecules, in which a moiety is cleavable or changeable in response to enzymes.[5] After enzymatic treatment, the molecular structure and property are drastically changed, triggering formation, breakdown, or transformation of assemblies.[6] The synthetic approaches, however, meet their own limitations in terms of laborious, multistep, or even low-yield synthesis and lack of generality for a single synthesis.[5,7] Alternatively, we present here a host–guest approach based on surfactant–cyclodextrin (CD) mixtures to sidestep the difficult synthesis and to fulfill generality to some extent.

Surfactants (or amphiphiles) are molecules with hydrophobic and hydrophilic moieties that can self-assemble into a variety of structures as driven by the hydrophobic effect.[8] CDs are donut-like oligosaccharides with a hydrophilic outer surface and a hydrophobic cavity.[9] CDs can form host–guest complexes with most surfactants in high binding constants by including hydrophobic moieties of surfactants into CD cavities. The resultant complexes are hydrophilic in their outer surface, and thus unable to assemble.[10] That is to say, surfactant assemblies will be destroyed upon the addition of CDs. On the other hand, amylase is a digestive enzyme that catalyses the breakdown of starch into sugars. α-Amylase can cleave α-1,4 linkages between glucose units of starch molecules including CDs, which will degrade CDs in two steps (ring opening and chain scission) giving glucose in the end (Fig. 1a).[11] Therefore in our host–guest approach, surfactants themselves are not responsive to α-amylase but their host–guest complexes with CDs might be. We expect that the addition of α-amylase to surfactant–CD mixtures will degrade CD molecules, release the included surfactant molecules, and trigger the self-assembly of the surfactant molecules. This expectation is testified in this work for three most fundamental, model assembly systems: monolayers, micelles, and vesicles (Fig. 1b–d). The host–guest

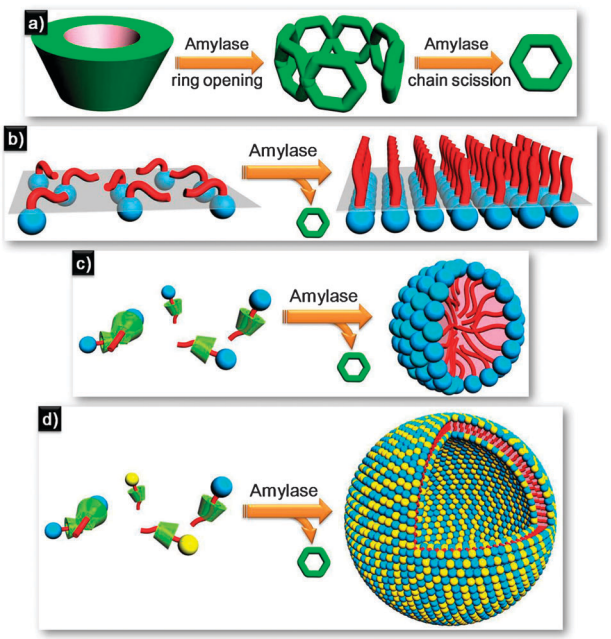

Fig. 1 Schematic illustrations of the degradation of β-CD by α-amylase (a), enzyme-triggered monolayer formation (b), enzyme-triggered micellization (c), and enzyme-triggered vesicle formation (d).

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approach is envisioned to be a general approach feasible for more complicated assemblies and broad applications.

Surfactant molecules in aqueous solution tend to enrich themselves at the air–water interface to form an adsorption monolayer.[8] Upon the addition of CD, surfactant molecules will be extracted from the monolayer to CD cavities, leading to breakdown of the monolayer and a water-like surface tension.[10] Here we attempt to test if the addition of α-amylase can restore the CD-destroyed surfactant monolayer. Among common native CDs (α-, β-, and γ-CD with 6, 7, and 8 glucose units, respectively), β-CD is selected because (1) its affinity to surfactants is higher than γ-CDs, (2) its degradation by α-amylase is much more efficient than α-CDs, and (3) it is the most economic one. Among numerous surfactants, TDPS, CTAB, SDS, and TritonX100 (please see the abbreviations‡) are chosen to represent four main kinds of surfactants, zwitterionic, cationic, anionic, and nonionic ones, respectively.

When the concentrations of the surfactants exceed or approach their critical micelle concentrations (CMCs), the surface tension of the solutions reaches a low value, $\sim$35 mN m^{-1} (Fig. 2, blue bars, where the concentration of TDPS, CTAB, SDS, and TritonX100 are 1, 1, 6, and 1 mM, respectively), indicating the formation of saturated adsorption monolayers. After the addition of excess β-CD, the surface tension increases by $\sim$20 mN m^{-1} (Fig. 2, red bars, where the concentrations of β-CD are 4, 4, 8, and 4 mM, respectively), suggesting the breakdown of the monolayers. In this situation, only a few surfactant molecules are sparsely distributed in the surface with their tails irregularly lying in the air phase (Fig. 1b left). Then after the treatment of 20 U ml^{-1} α-amylase, β-CD molecules are degraded and the surface tension returns to a low value, almost the same as that before the CD-addition (Fig. 2, green bars), implying the recovery of the adsorption monolayers. Please note that this high dosage (20 U ml^{-1}) was chosen to get a dramatic change within hours and a complete degradation of β-CD in 24 hours. The degradation of β-CD by α-amylase clearly releases surfactant molecules and triggers the formation of the adsorption monolayers. One argument is that the decrease of the surface tension may come from the amphiphilicity of α-amylase itself. This possibility is ruled out by a water-like surface tension of a surfactant-free β-CD–α-amylase solution. It is noteworthy that α-amylase is effective for all the four kinds of surfactants.

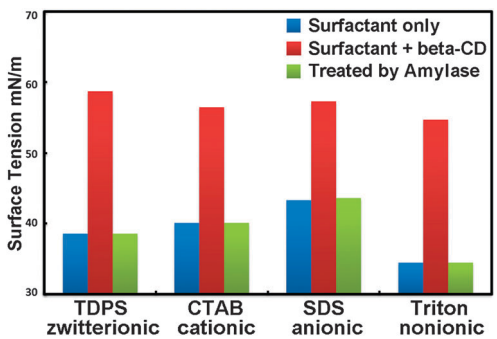

Fig. 2 A column diagram for the solution surface tensions of surfactants, surfactant–CD mixtures, and α-amylase-treated surfactant–CD mixtures (TDPS 1 mM, β-CD 4 mM; CTAB 1 mM, β-CD 4 mM; SDS 6 mM, β-CD 6 mM; TritonX100 1 mM, β-CD 4 mM; the dosage of α-amylase is 20 U ml^{-1} in all cases).

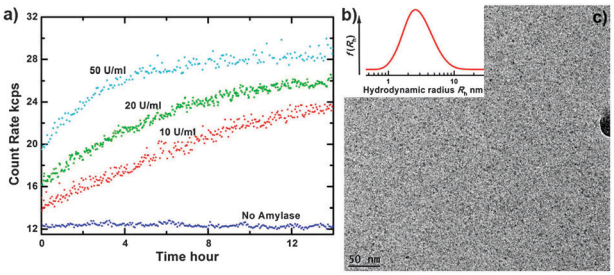

Fig. 3 Variation of the scattering intensity of the TDPS–β-CD (5 : 8 mM) solution since the addition of different dosages of α-amylase (a). At T = 24 hours, the DLS result (b) and Cryo-TEM image (c) of the micelles formed in the solution.

Above the CMC, surfactant molecules can assemble into micelles,[12] which can be, as being well documented,[10] dissembled by the addition of CDs. Here the restoring effect of α-amylase on surfactant micelles is tested in a TDPS–β-CD system. A 5 mM TDPS (CMC $\sim$ 0.3 mM) aqueous solution is predominated by plenty of micelles. After the addition of 8 mM β-CD, the solution scattering is notably reduced and no particles larger than 1 nm can be detected by dynamic lighter scattering (DLS), implying disassembly of the TDPS micelles. Different dosages of α-amylase are applied to the TDPS–β-CD (5 : 8 mM) solution, the scattering of which is recorded in real-time (Fig. 3a). In the very beginning of α-amylase addition (T = 0 hour), the scattering intensity is elevated to different extent depending on the α-amylase dosage because α-amylase molecules themselves can scatter light. Then with time passing, the scattering intensity of the α-amylase-free solution is constantly low, whereas that of the α-amylase-loaded solutions gradually increases, suggesting the formation of assemblies. The intensity increases faster for a higher dosage of α-amylase: for the 50 and 20 U ml^{-1} dosages, the intensity reaches a plateau after 6 and 12 hours, respectively; as for the 10 U ml^{-1} dosage, the intensity does not yet reach any plateau in 14 hours. At T = 24 hours, the size of the assemblies is determined by DLS (Fig. 3b). The size distributions for different dosages of α-amylase are almost the same, a relatively narrow distribution with an averaged hydrodynamic radius of $\sim$2 nm, in line with a typical size of spherical micelles. The assemblies are visualized by cryogenic-transmission electron microscopy (Cryo-TEM, Fig. 3c), where small spherical structures prevail the whole image, confirming the existence of micelles. Taken together, we can say that the addition of α-amylase to the TDPS–β-CD solution will degrade β-CD molecules over time and will consequently release TDPS molecules to form micelles (Fig. 1c).

Vesicles are a kind of fundamental yet indispensable self-assembled structures for *in vitro* studies mainly because of their bilayer membranes and inner water pools.[13] Unlike micelles, vesicles are usually formed by double-chain surfactants or lipids, rather than single-chain surfactants. It has been reported that CDs can neither bind with double-chain surfactants or lipids effectively nor affect their vesicles significantly.[14] Here we resort to cationic–anionic surfactant (single-chain) vesicles. In this kind of systems,[15] the electrostatic repulsion between surfactant ionic headgroups are greatly compromised by the oppositely charged surfactant, enabling formation of vesicles, while the two single-chain surfactants can still bind with CDs.[16]

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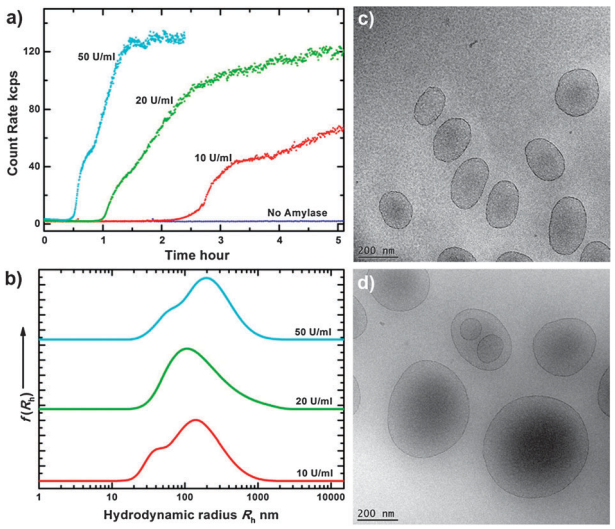

Fig. 4 Variation of the scattering intensity of the SDS–DEAB–β-CD (0.4 : 0.6 : 8 mM) solution since the addition of different dosages of α-amylase (a). At T = 24 hours, the DLS result (b) and Cryo-TEM images (c, d) of the vesicles formed in the solution.

Specifically, the pair of SDS and DEAB (please see the abbreviations) is chosen. The SDS–DEAB (0.4 : 0.6 mM) solution is dominated by vesicles, which will fully vanish upon the addition of 8 mM β-CD. Then α-amylase is applied to the SDS–DEAB–β-CD (0.4 : 0.6 : 8 mM) solution at three different dosages, and the solution scattering is followed in real-time (Fig. 4a). At T = 0 hour, the scattering intensity increased a little due to the presence of α-amylase. With time passing, the scattering intensity of the α-amylase-free solution stays unchanged, while that of the α-amylase-loaded solutions experiences drastic increases (up to 30 times), indicating the formation of large assemblies that can strongly scatter light. The scattering curves are of irregular fashions, where a general trend is that the intensity remains constant for a while, subsequently goes through a fast-increase stage and a slow-increase stage, and finally reaches a plateau of maximum. The irregularity reflects a complicated relation between the degradation reaction and the scattering intensity, which will be investigated in detail in further work. A solution with a higher α-amylase dosage is of a much shorter lifetime for the scattering increase process. For the 50 U ml^{-1} dosage, it only takes 1.5 hours for the intensity to reach the maximum, while for the 20 U ml^{-1} dosage, it takes more than 5 hours. At T = 24 hours, the size distributions of the assemblies are determined to be broad distributions with peaks ~100 nm (Fig. 4b), in coincidence with a typical size of vesicles. In the Cryo-TEM images (Fig. 4c and d), there are many hollow spherical or ellipsoidal structures ranging from 100 to 300 nm, as well as some nested shell structures, proving the formation of vesicles. It is clear that α-amylase can trigger the assembly of vesicles in the SDS–DEAB–β-CD system (Fig. 1d).

We constructed enzyme-responsive model self-assembly systems in virtue of a surfactant–CD based host–guest approach. It is found that surfactants themselves are not responsive to α-amylase, whereas surfactant–CD complexes are. The addition of α-amylase to surfactant–CD systems will degrade the CD molecules, release surfactant molecules from CD cavities, and consequently trigger the self-assembly of the surfactant molecules. According to this simple principle, enzyme-triggered self-assembly of monolayers, micelles, and vesicles is realized. This principle is valid for a range of surfactant systems (such as zwitterionic, cationic, anionic, nonionic ones) and mixed surfactant systems. It is envisioned that the present host–guest approach is a general approach feasible for more complicated assemblies with broad applications. Moreover, it is worthy to mention that the abnormal increase of α-amylase is intimately associated with acute pancreatitis,[17] thus the current α-amylase-triggered self-assembly might be of potential use in their early detection and clinical treatment.

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Notes and references

‡ *Abbreviations.* TDPS: tetradecyl dimethyl ammonium propane sulfonate; CTAB: cetyl trimethyl ammonium bromide; SDS: sodium dodecyl sulfate; TritonX100: polyoxyethylene octyl phenyl ether; DEAB: dodecyl triethyl ammonium bromide.

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