Supporting Information

1 materials and methods

1.1 Materials

Amylose, acetic anhydride, propionic anhydride, butyric anhydride, propofol, umbelliferone, and DMPC were purchased from Sigma-Aldrich Trading Co. Ltd. A dialysis bag with a cut-off molecular weight of 500 Da was purchased from MYM Biological Technology Co. Ltd. Other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd.

1.2 Preparations and characterizations of AAHAs.

1.2.1 Preparations and characterizations of acylated amylose.

Acylated amylose were prepared with the modified method reported by Asim et al. [1] One gram of amylose was dissolved in a reaction flask dissolved by 50 mL of DMSO. The system was heated to 80 °C for 2 h to pre-activate the system. 1-Methylimidazole (0.2 mL) and different amounts of anhydride were added drop-wise, and the reaction continued with stirring for 4 h to ensure reaction equilibrium. A large volume of ethanol was added in the reaction mixture to coagulate the content. The coagulation was centrifuged and washed with methanol three times. Acetylated amylose helix was obtained by drying the precipitate at 50 °C for 24 h. The obtained powders (100 mg) were dissolved in 10 mg propofol, and 4 mL pure water was injected in under vigorous stirring conditions. After 30 min, the propofol-loaded acylated amylose helices were prepared. The propionylated and butyrylated amylose helices were prepared by using the propionic anhydride and the butyric anhydride. Other molecules loaded acylated amylose helices were prepared using the same method mentioned above.

To determine the chemical structure of native and acylated amylose helices, the FTIR spectra were recorded by FTIR instrument (Shimadzu Corporation, Japan) at a resolution of 4 cm^{-1} and wave number ranging between 400 and 4000 cm^{-1}. Moreover, their ^{1}H NMR spectra were also recorded using an Avance 300 MHz super-

conducting Fourier digital NMR spectrometer (Bruker Corporation, Switzerland) by dissolved those amylose derivatives in DMSO-d6. The DS were determined by titration method.[1] The crystalline morphology of native and acylated amylose helices were verified by measuring the XRD using an X'Pert PRO X-ray diffractometer (Panalytical, Holland). The range of diffraction angle (*2θ*) was from 5 to 40°. As a control, the acylated amyloses without loading molecules were characterized.

1.2.2 Preparations and characterizations of AAHAs.

Molecules-loaded AAHAs were prepared as follows: 100 mg of molecules-loaded with acetylated amylose helix were dissolved in 10 mL pure water. The solution was stirred at 50 °C for approximately 30 min. The system was cooled to room temperature to obtain stable AAHAs. To remove the free molecules in the solution, the obtained mixture was dialyzed in a large volume of 50% (w/v) PEG 2000 solution at 37 °C for 24 h.

To confirm the molecules wrapped in AAHAs, the crystalline morphologies of the AAHAs loaded with and without molecules of AAHAs were measured by XRD. The FTIR and ^{1}H NMR spectra were also measured to verify the molecules loading in AAHAs. The morphology of AAHAs was measured using transmission electron microscopy (TEM) (Hitachi, Japan). Their size distribution was measured by utilizing a Malvern Nano-zs 90. The molecule loading efficiency (MLE) of the amylose was calculated as follows:

$$MLE\ \% = \frac{M_{molecules}}{M_{nanoclusters}} \times 100\% \qquad (2\text{-}1)$$

where $M_{nanoclusters}$ is the total content of AAHAs, and $M_{moulecules}$ is the content of encapsulated molecules. The $M_{nanoclusters}$ is measured by freeze drying the AAHAs solution. The encapsulated molecules were released by dissolved in strong alkaline solution. The mixture solution was precipitated by adding a large volume of absolute ethanol and the drug concentration in supernatant was measured.

1.3 Characterizations of triggered release of the AAHAs.

As a typical example, we prepared iodine-loaded AAHAs through the above-mentioned procedure. The final concentration of 50 mM alcohol solution of propofol was added to trigger the release of iodine. We then determined the concentration of wrapped iodine by measuring the absorption value at 620 nm. The triggered-release duration was defined as the amount of time required for the release of 80% of the loaded molecules. We also investigated the factors influencing the molecule-triggered release, such as temperature (0 °C to 50 °C), pH (3 to 10), and NaCl concentration (0 mM to 60.0 mM). To achieve the controllable duration time of the molecule-triggered release, we added different amounts of 0.5 M propofol solutions (0 mL to 1.0 mL) to 1.0 mL of iodine-wrapped AAHAs and diluted the resulting solution with pure water to 2.0 mL. The iodine concentration was determined continuously. Herein, the duration time is defined as the time from the start of addition to 80% triggered release of AAHAs.

The sequential molecule-triggered release attribute of the AAHAs was confirmed as follows. First, we prepared salicylic acid wrapped AAHAs as described above, then added 100 μL of 0.5 M propofol alcoholic solution to the AAHAs under vigorous stirring. After 5 min, we placed the solution in a dialysis bag with a cut-off molecular weight of 500 Da and suspended the dialysis bag in a release volume of 200 mL PEG2000 (50% w/v) aqueous solution at 37 °C. At scheduled intervals, we collected 2 mL of the release medium for assay. We then quantified the concentration of salicylic acid by UV/Visible (UV/Vis) spectrophotometry at 527 nm with $FeCl_3$ as the chromogenic agent.[2] Similarly, we added 100 μL of 0.5 M umbelliferone alcoholic solution in the inner liquid to trigger the propofol release from the AAHAs. The propofol concentration was determined by UV/Vis spectrophotometry at 273 nm. Finally, we used the same amount of DMPC to release the umbelliferone. The fluorescence intensity at 453 nm was employed to determine the concentration of umbelliferone at the excitation wavelength of 327 nm.

1.4 Controlled molecule-triggered mechanism.

To determine the binding energies of acetylated amylose helices to different loaded molecules (iodine, salicylic acid, propofol, umbelliferone, and DMPC), free bonding energies between these molecules and helices were calculated by molecules dynamic (MD) simulation. In detail, a series of 21-mer amylose chains acetylated at O6 with the DS = 0.45 were modeled based on AmberTools14 because the C6OH is more reactive than others. Acylation more readily occurred than the C2 and C3 because of steric hindrance.[3] The topology files of iodine, propofol, salicylic acid, and umbelliferone were generated by ATB server.[4] The DMPC model was proposed by Poger et al.[5] All the bond lengths were constrained by the LINCS algorithm.[6] The simple point charge (SPC) water model was used as the solvent.[7] These MD simulations were carried out with GROMACS version 4.6 by using the GLYCAM-06 force field.[8] The temperature was maintained at 300 K, and the pressure was maintained at 1.0 bar.

Initially, these molecules loaded acetylated helices were formed by simulating the mixture of these molecules and the acetylated amylose together in vacuum for 100ns. Then, these helical structures were dissolved in water and simulated for 200 ns to obtain stable hydrated structures. The free bonding energies were calculated by the potential of mean force (PMF) through the umbrella sampling approach. Then, on the basis of the PMF results, we computed for the binding energies between propofol and different molecules wrapped in acetylated amyloses. In the process, we placed the acetylated amylose helix in a rectangular box of dimensions 14 nm × 7 nm × 7 nm, with the helical axis along the *x* dimension. The box was then filled with water molecules. The atoms of the wrapped molecules were restrained with a force constant of 1000 kJ mol^{-1} nm^{-2}, which moved at a speed of 10 nm per ns in the direction of the x axis for 500 ps. Then, we obtained 50 window points spaced by 0.1 nm and performed 20 ns of simulation for each window. The PMFs were reconstructed using the weighted histogram analysis method.[6] The statistical errors were also calculated.

To clarify the molecule trigger mechanism for AAHAs, we measured the particle sizes and fluorescence spectral changes in AAHAs during the molecule trigger. This step was included to reflect the microstructural reconstruction. The salicylic acid and propofol were selected as a pair of trigger-effectors because salicylic acid holds the fluorescence activity, whereas the propofol possesses none and could thus avoid fluorescence interference. More importantly, the molecule-triggered process of this system can last about 2 h, which was a sufficient duration for measuring the particle sizes and fluorescence changes. Specifically, 100 μL of 10 mM propofol solution was added in 2 mL of 1% (w/v) salicylic acid wrapped AAHAs by dissolving in the pH 7.4 PBS solution. For the control group, the salicylic acid-loaded AAHAs were dissolved in 2 mL of pH 10 buffers because the amylose helix can unfold and decrease in size under alkaline conditions.[9] The fluorescence spectra of salicylic acid and the sizes of the AAHAs were measured immediately every 5 min for 2 h.

The triggered release process of the AAHAs was also simulated by MD simulation. In particular, a salicylic acid wrapped, acetylated amylose helix was dissolved in a 7 × 7 × 7 nm cubic box filled with propofol solution. The system was simulated for 100 ns to model the release of salicylic acid molecules from AAHAs. The developments of the distance between the salicylic acid and helix, the number of intramolecular hydrogen bonds, and the radius of rotation were calculated during the simulation.

2. Characteristics of acetylated amylose.

2.1 Confirming the acetylation

FTIR and ^{1}H NMR spectra were employed to verify acylation of amylose helices. FTIR spectra of the native and acetylated, propionylated and butyrylated amylose helices are displayed in Figure S1a. In the spectra of native amylose helix, the peaks at 1158, 1081, and 1014 cm^{-1} can be attributed to the C–O band stretching. Additionally, the absorption bands at 928.8, 860.7, 764.6, and 575.1 cm^{-1} can be attributed to the anhydroglucose ring stretching vibrations. A broad band at 3398.1 cm^{-1} appeared because of the –OH groups. The FTIR spectra of acetylated,

propionylated, and butyrylated amylose helices showed new characteristic bands at 1730 and 1201 cm^{-1}, assigned to the carbonyl C=O vibration from saturated ester. Furthermore, the intensity of the peak at 3398 cm^{-1}, which was derived from the –OH groups weakened after acylation, indicated that hydroxyl groups participated in the reaction. This result further suggested the esterification between amylose and propionic anhydride.

The FTIR spectra could not fully distinguish among the acetylated, propionylated, and butyrylated amylose helices; hence, ^{1}H NMR spectra of the samples were obtained. In the spectra of the native amylose helices, the characteristic peaks correspond to those of the proton bound to the C1 of glucopyranose units at 5.10 ppm; the proton bound to C2–6 between 3.8–3.2 ppm; and the hydroxyl groups at 5.53, 5.41, and 4.60 ppm (Figure S1b). Meanwhile, the resonation of the methyl hydrogen was found at 2.2 ppm in the ^{1}H NMR spectra of the propofol-loaded, acetylated amylose helix, and the intensity of the hydroxyl groups of the glucopyranose units weakened. This aspect indicated the successful esterification between amylose and acetic anhydride. Additionally, acetylated amylose helix held characteristic peaks of amylose. Peaks at 2.33 and 1.04 ppm were due to the H of –CH2 and –CH3 in the ^{1}H NMR spectra of propionylated amylose helix. We concluded that the esterification between amylose and propionic anhydride occurred. The ^{1}H NMR spectrum of butyrylated amylose helix showed that the absorption peak at 0.77 ppm caused by $–CH_3$ was observed. The peaks at 1.43 and 2.17 ppm of the spectrum were derived from the methylene group (CH_2) and the methylene group adjacent to the carbonyl group ($CH_2C=O$), respectively. The data clearly revealed the occurrence of reaction between the amylose and butyryl groups.

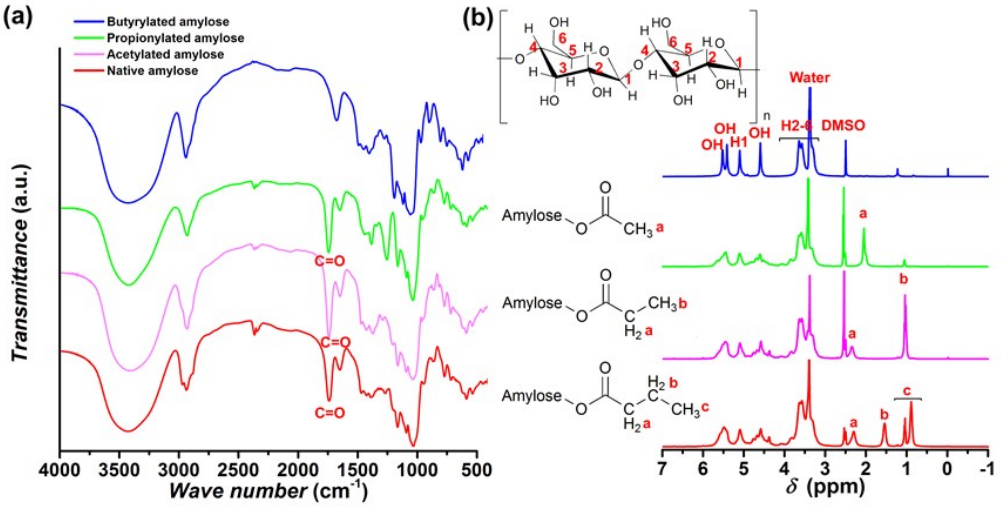

Figure S1. The FTIR (a) and ^{1}H NMR (b) spectra of native amylose, acetylated, propionylated and butyrylated amylose helices.

2.2 Optimization of the type of acylation

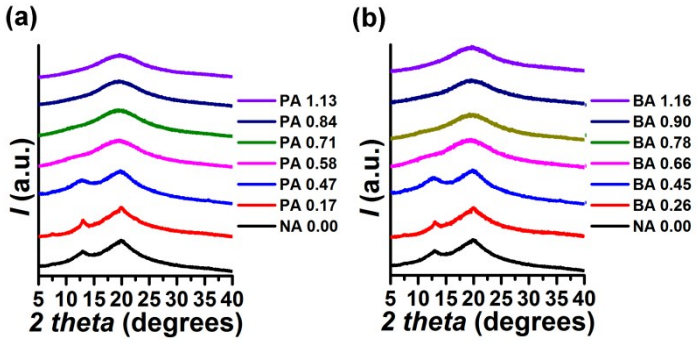

Figure S2. The XRD patterns of propionylated amyloses (PA) (b) and butyrylated amyloses (BA) (c) with different DS.

3 The structure and stabilty of AAHAs

3.1 The XRD of different molecule-loaded AAHAs

FTIR patterns of the molecule-loaded with AAHAs were shown in Figure S3a. The characteristic bands of the loaded molecules were rarely detected caused by the shielding effects of AAHAs, suggesting that these molecules were loaded in the cavity of the amylose helix. To further elucidate the crystal forms of the salicylic acid, propofol, umbelliferone, and DMPC-loaded AAHAs. The XRD spectra of these AAHAs are mearsured (Figure S3b). The XRD peaks of the molecules-loaded

AAHAs were approximately 13° and 20°, suggesting the formation of a V-type helix. [10] compared with the pure molecules, the XRD spectra of molecules loaded AAHAs do not present any characteristic peaks of free molecules, indicating these molecules was sucessfully loaded in AAHAs.

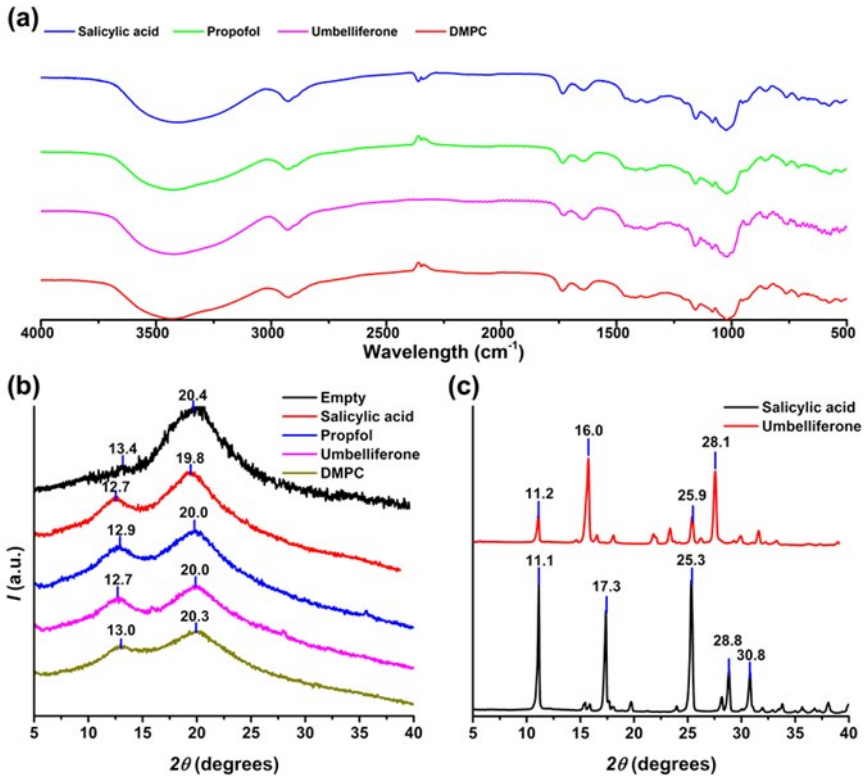

Figure S3. FTIR (a) and XRD (b) patterns of AAHAs with loading salicylic acid, propofol, umbelliferone, and DMPC. The XRD patterns of pure salicylic acid and umbelliferone (c).

3.2. The stability of AAHAs with different DS

The iodine-loaded amylose helix provides useful information on the extension of helical segments in solution. As shown in Figure S4, The blue value decreased with an increase in the DS showing that the content of continuous helical segments of acetylated amylose decreased with an increase in the DS. Specially, when DS was bigger than 1.16 the solution almost became colorless indicating the helical being descried completely. On the other hand, the stability of the AAHAs was mainly dependent on the DS. Native and lower DS acetylated AAHAs were gradually subside precipitated over 24 hours, but the acetylated amylose nanoclusters appeared to be no

change due to their good solubility. This indicated moderate acylation of AAHAs could improve the stability in water.

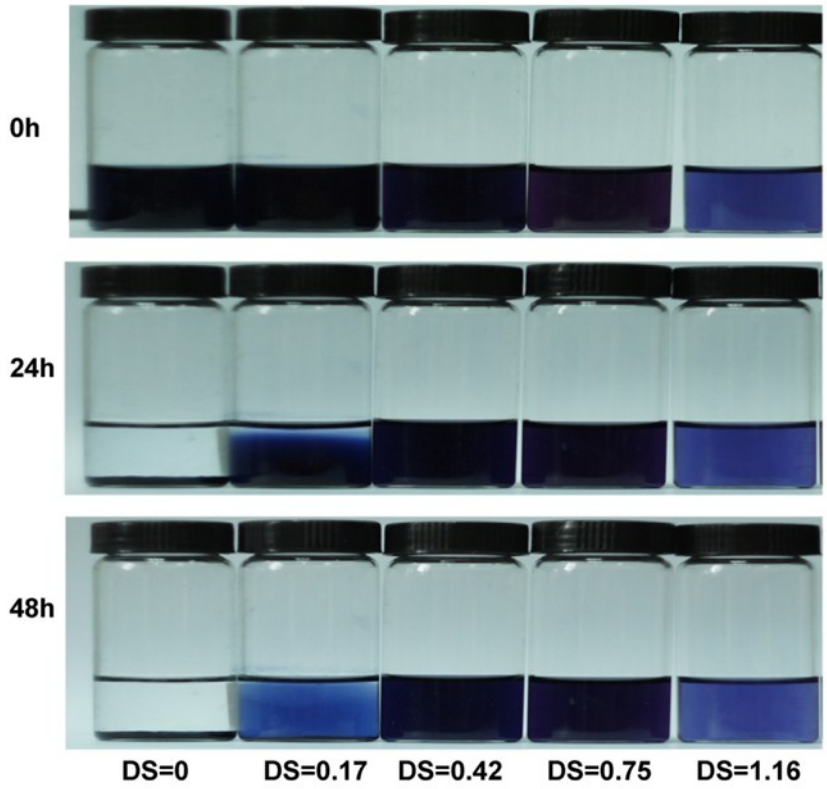

Figure S4. The photos of iodine-loaded AAHAs with different DS during standing for 24h.

4 Characteristics of AAHAs.

4.1 Morphology of AAHAs

Figure S5 showed the TEM photograph of empty and propofol loaded AAHAs. The empty AAHAs were in the form of flocculent particles. They were difficult to aggregate into regular nanospheres, and the distribution of particle size was polydisperse. The propofol-loaded AAHAs have homogeneous size, and the particle consists of smaller solid block particles. Due to the interhelical hydrogen bonds, the amylose helices were inclined to form self-aggregations.[11] Thus, the solid block particles might be suggested to the single helix of propofol loaded AAHAs.

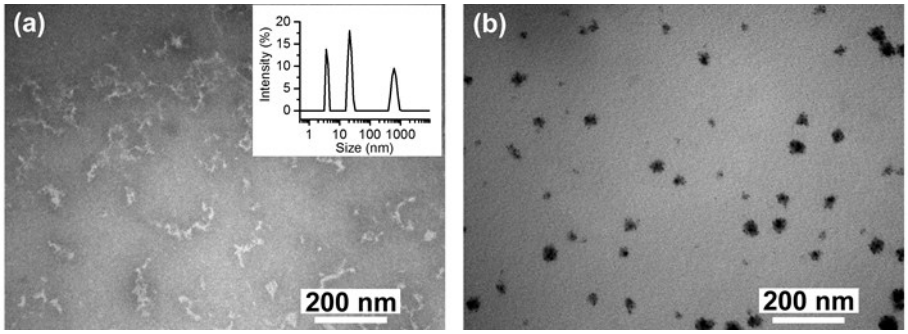

Figure S5. TEM (negatively stained) photograph of empty AAHAs (a) and the size distribution in the illustration. TEM photograph of propofol-loaded AAHAs (b).

4.2 The stability of acetylated AAHAs

In order to determinate the viability of AAHAs in media of biological relevance. The sizes of propofol-loaded acetylated AAHAs were measured in different pH, temperatures, concentration of salts, and cell culture media. As shown in Figure S6a-d, the AAHAs were scarcely changed in physiological pH. The size was about 60 nm under the physiological temperature and increases with the further rise of temperature. In different concentrates of NaCl solution, the AAHAs presented similar sizes. The AAHAs kept stable in cell culture media for 2 weeks and then were degraded gradually.

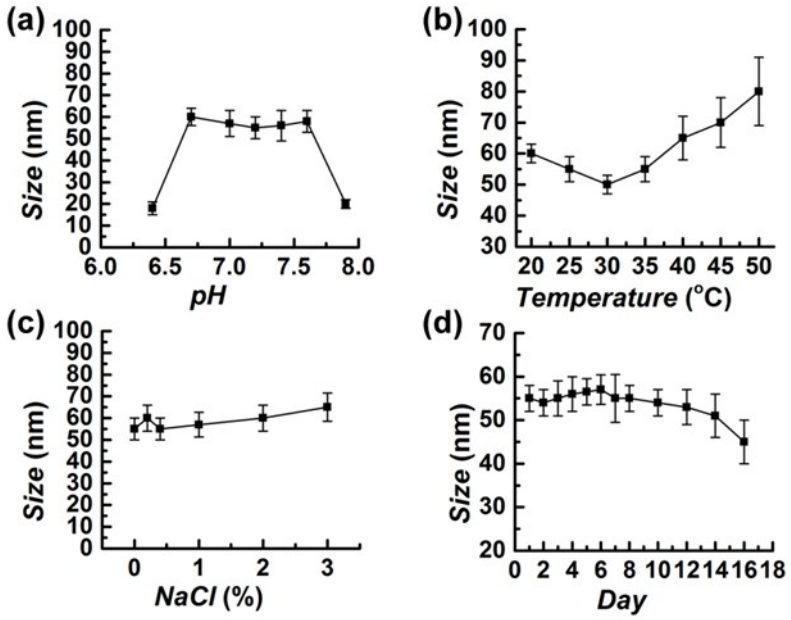

Figure S6. The size changes of propofol-loaded AAHAs in different pH (a), temperatures (b), concentrations of salts (c), and cell culture media for two weeks (d).

4.3 The sizes, PDIs and MLEs of AAHAs with different molecules

In the different molecules-loaded AAHAs, the sizes and PDIs were very slightly different (Figure S7a, b), but their MLEs showed distinct dissimilarities (Figure S7c).

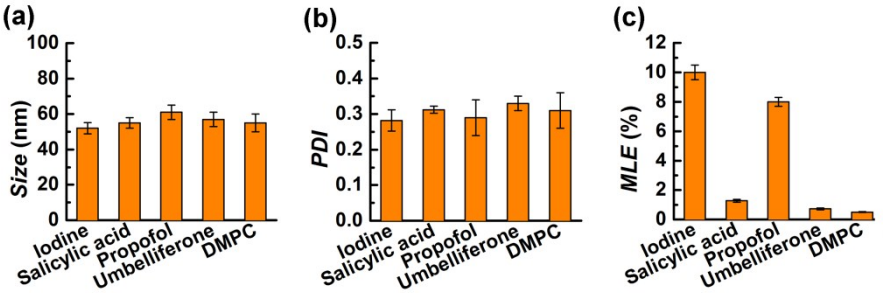

Figure S7. The sizes (a), PDIs (b), and MLEs (c) of acetylated AAHNCs with DS = 0.42 loading different molecules loading iodine, salicylic acid, propofol, umbelliferone, and DMPC.

5. Triggered-release characteristics of AAHAs

5.1 The influence of surrounding factors on the triggered-release of AAHAs

To strengthen the control of the triggered-release system, we investigated the influence of the DS and surrounding factors, such as pH, ionic strength, and temperature. The process of iodine-loaded AAHNCs triggered by propofol was selected as a typical system, in which the wrapped iodine exhibited a blue color but the free iodine did not. In Figure S8a–d, the triggered-release durations of the AAHAs under different DSs were variable. For the AAHAs with low DS, the triggered-release duration was extremely long, and the duration decreased as the DS increased. This trend can be ascribed to the loosening of structure caused by acylation. The time period under acidic conditions slightly changed but decreased as pH > 7 because the intermolecular hydrogen bonds were partly destroyed in basic solution. Moreover, the

duration did not obviously change in different ionic strengths but increased with temperature.

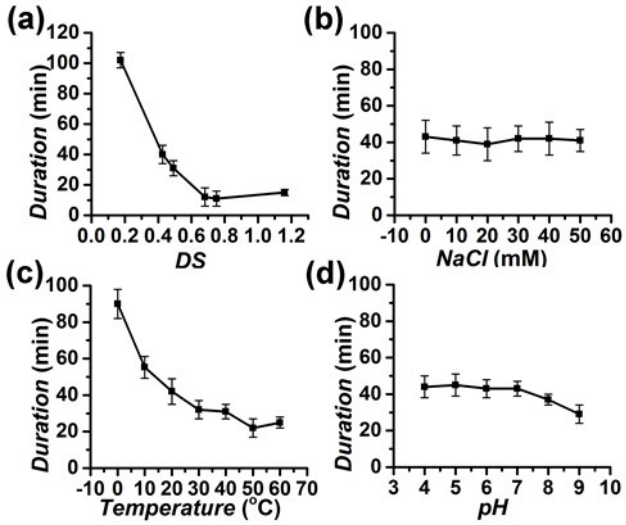

Figure S8. Duration times of the triggered release of AAHAs with different DSs (e), salt ionic strength (f) temperature (g), and pH (h).

5.2 Controllability of the triggered-release of AAHAs

As shown in Figure S9a, the iodine-loaded AAHAs could be triggered release by salicylic acid. When adding $FeCl_3$ solution, the triggered release process could be blocked and the concentration of free iodine almost unchanged anymore because of the form of salicylic acid-Fe^{3+} complexes.[12] Thus, the triggered release AAHAs could be blocked by adding blocker.

According to the results of free bonding energy calculation by MD simulation that the bonding free energy of salicylic acid is less than that of propofol, and the free-energies ΔG from salicylic acid AAHAs to propofol AAHAs is negative. So we can conclude that the transformation from salicylic acid-loaded AAHAs to propofol-loaded AAHAs would happened spontaneously. In other words, salicylic acid in AAHAs may be triggered by propofol. In order to testify this idea, fluorescence-emission of salicylic acid-loaded AAHAs in aqueous solution was measured. The results (Figure S9b) indicated that when the salicylic acid was wrapped in AAHAs, its fluorescence would be quenched and this phenomenon had also been observed in the cyclodextrin complex.[13] As propofol was added into the solution, the quenching

effects disappeared which demonstrates the salicylic acid-loaded AAHAs could be triggered by propofol.

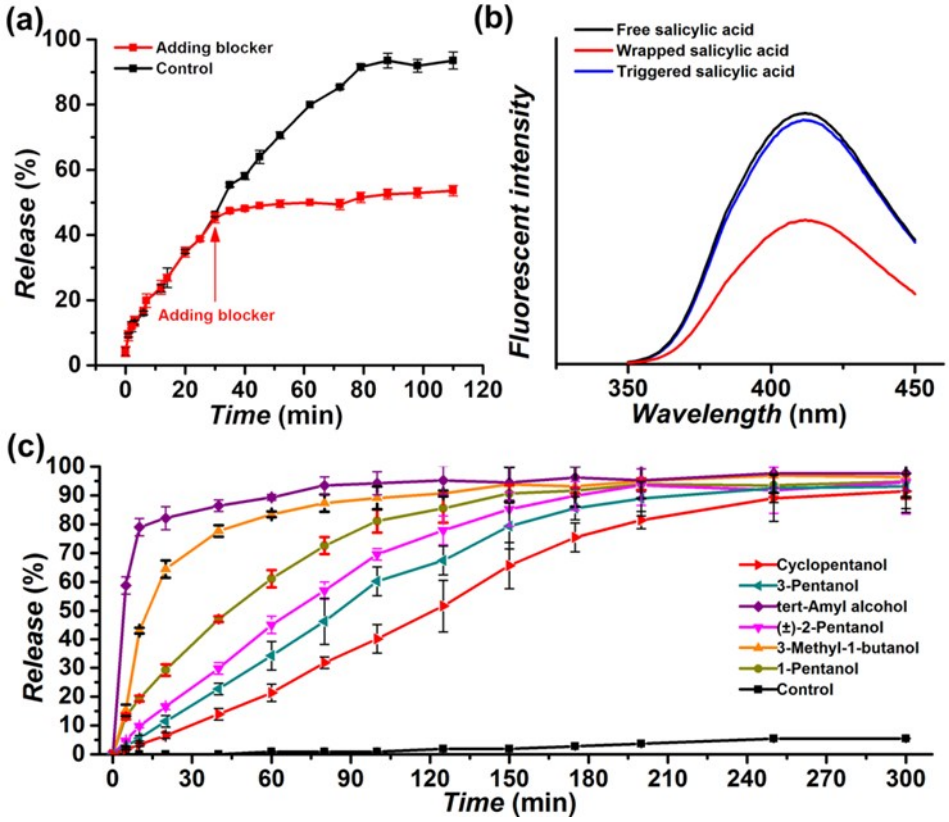

Figure S9. The iodine-loaded AAHAs triggered by salicylic acid and blocked by adding $FeCl_3$ solution (a). The fluorescence intensity changes of salicylic acid-loaded AAHAs triggered by propofol after 2h (b). The triggered release curves of iodine-loaded AAHAs by cyclopentanol, 3-pentanol, (±)-2-pentanol, 1-pentanol, 3-methyl-1-butanol, tert-methyl alcohol (c) in sequence.

The triggered-release duration of iodine-loaded AAHAs can also be controlled by adding different hydrophobic molecules. For instance, as shown in Figure S9c, the iodine-loaded AAHAs can be triggered by 300mM five-carbon alcohol with different duration time from 5 to 300 min. The sequence arranged in order of duration decrease is cyclopentanol > 3-pentanol > (±)-2-pentanol > 1-pentanol > 3-methyl-1-butanol > tert-methyl alcohol. This indicate the duration time of the triggered release could be adjusted by adding different molecules.

5. 3 Molecule triggered-release mechanism

The molecule-triggered-release process was studied by MD simulations to confirm the above inference. As shown in Figure S10a, the salicylic acid molecules were gradually replaced by the propofol molecules during the simulation. All of the salicylic acid molecules were released after 100 ns of simulation. Meanwhile, the hydrophobic cavity of the helix was fully occupied by propofol. The change in distance between the salicylic acid and amylose helix clearly reflected this process (Figure S10b). The distance increased as the trigger was applied. However, the number of hydrogen bonds and the gyration radius of the helix remained unaltered during the entire process (Figure S10c,d). This result implies the retention of the helical structure throughout all of the molecule-triggered-release steps. Specifically, the wrapped salicylic acid molecules were pushed outward from the helical cavity by propofol. Similar results were also found with AAHAs loaded with other molecules.

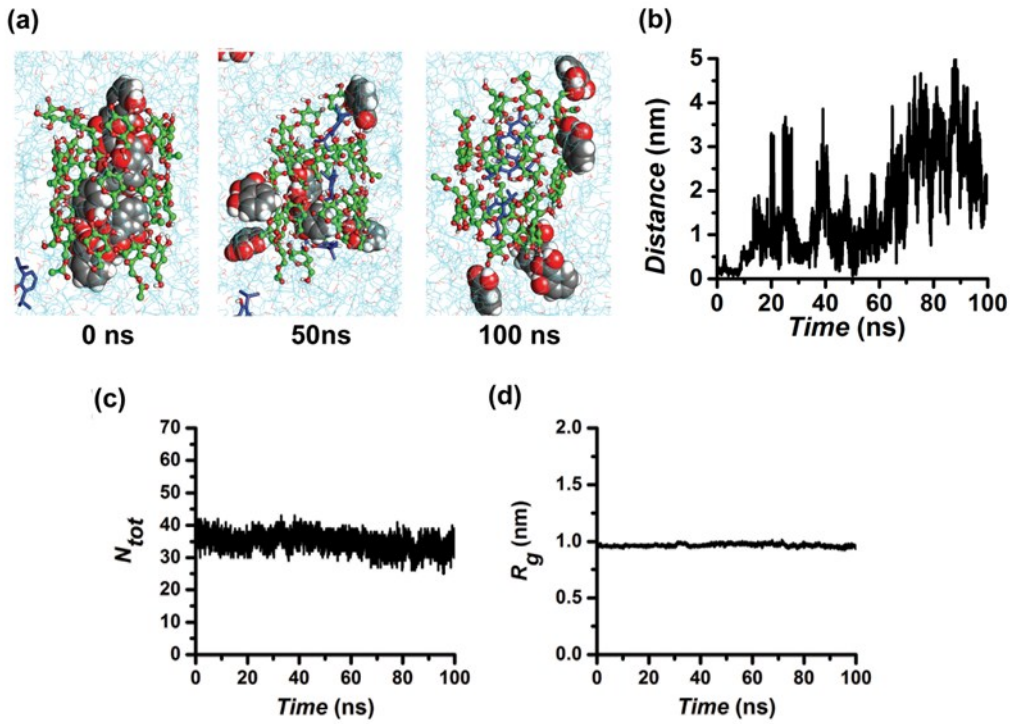

Figure S10. The MD simulation snapshots of salicylic acid-loaded AAHAs triggered by propofol (a). The development of the distance change between the salicylic acid

and amylose helix (b).The development of interhelical hydrogen bonds (c), and radius of gyration (d) of amylose helix during the 100ns of triggered-release simualtion.

Referances

[1] S. Garg, A. K. Jana, *Carbohydr. Polym.*, **2011**, *83*, 1623.

[2] J. M. Meyer, P. Azelvandre, C. Georges, *Biofactors,* **1992**, *4*, 23.

[3] A. F. Rogovin, Z. A. Rogovin, *Polymer Science U.S.S.R.,* **1963**, *4*, 1189.

[4] A. K. Malde, L. Zuo, M. Breeze, M. Stroet, D. Poger, P. C. Nair, C. Oostenbrink, A. E. Mark, *J. Chem. Theory Comput.,* **2011**, *7*, 4026.

[5] D. Poger, W. F. Van Gunsteren, A. E. Mark, *J. Comput. Chem.,* **2010**, *31*, 1117.

[6] J. Y. Xie, G. H. Ding, M. Karttunen, *Biochim. Biophys. Acta-Biomembr.,* **2014**, *1838*, 994.

[7] D. M. Ferguson, *J. Comput. Chem.,* **1995**, *16*, 501.

[8] B. Hess, C. Kutzner, D. van der Spoel, E. Lindahl, *J. Chem. Theory Comput.,* **2008**, *4*, 435.

[9] M. E. Heineck, M. B. Cardoso, F. C. Giacomelli, N. P. da Silveira, *Polymer,* **2008**, *49*, 4386.

[10] C. Heinemann, F. Escher, B. Conde-Petit, *Carbohydr. Polym.*, **2003**, *51*, 159.

[11] W. C. Obiro, R. S. Sinha, M. N. Emmambux, *Food Rev. Int.,* **2012**, *28*, 412.

[12] J. M. Meyer, P. Azelvandre, C. Georges, *Biofactors* **, 1992**, *4*, 23.

[13] F. E. Oddy, S. Brovelli, M. T. Stone, E. J. Klotz, F. Cacialli, H. L. Anderson, *J. Mater. Chem.,* **2009**, *19*, 2846.