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Self-Assembly of Amphiphilic Anthracene-Functionalized β-Cyclodextrin (CD-AN) through Multi-Micelle Aggregation

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Multi-micelle aggregation (MMA) mechanism is widely acknowledged to explicate large spherical micelles self-assembly, but the process of MMA during self-assembly is hard to observe. Herein, a novel kind of strong, regular microspheres fabricated from self-assembly of amphiphilic anthracene-functionalized β-cyclodextrin (CD-AN) via Cu(I)-catalyzed azide-alkyne click reactions is reported. The obtained CD-AN amphiphiles can self-assemble in water from primary core–shell micelles to secondary aggregates with the diameter changing from several tens nm to around 600–700 nm via MMA process according to the images of scanning electron microscopy, transmission electron microscopy, and atomic force microscopy as well as the dynamic light scattering measurements, followed by further crosslinking through photodimerization of anthracene. What merits special attention is that such photo-crosslinked self-assemblies are able to disaggregate reversibly into primary nanoparticles when changing the solution conditions, which is benefited from the designed regular structure of CD-AN and the rigid ranging of anthracene during assembly, thus confirming the process of MMA.

1. Introduction

Amphiphiles self-assembly has attracted decades of attention[1–3] thanks to the extensive uses of supramolecular nanotechnology in the science of biomimetic materials, drug delivery systems, photoelectric materials, etc.[4,5] Amphiphilic molecules, which have hydrophilic head group and hydrophobic tails, can self-assemble into ordered structures by non-covalent interactions,[6–10] and the morphologies of which varied from simple spheres, cylinders, vesicles, to many hierarchial or complex,[3,11,12] while their specific structures contribute to the desired properties. Multi-micelle aggregation (MMA) mechanism reported frequently, is well known to explain amphiphile assembly-disassembly process for understanding the assembly formation mechanism and designing particular structures, but the primary state of MMA is hard to capture.[13–15]

Among various efficient and promising amphiphilic assemblies in supramolecular nanotechnology, cyclodextrin (CD) which possesses a hydrophilic exterior surface and hydrophobic interior cavity, expands the design and

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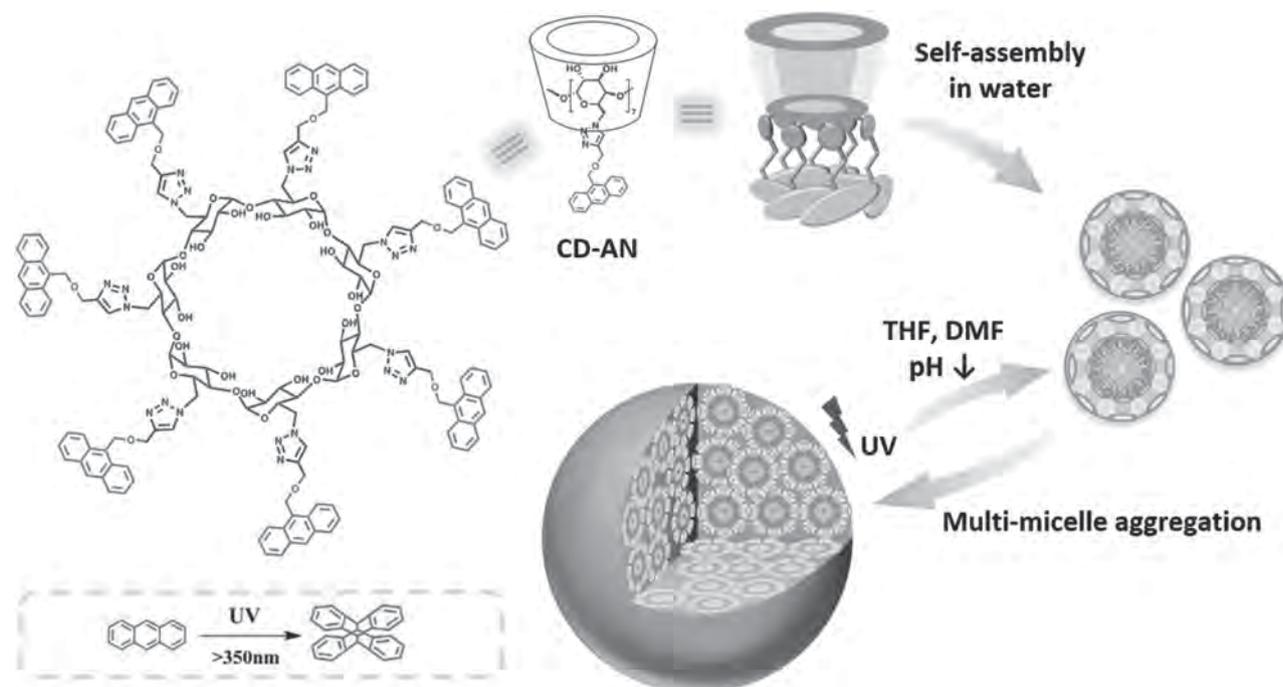
■ *Scheme 1.* Formation, self-assembly, and disaggregation process of CD-AN.

applications of supramolecular structures.[16–19] Self-assembly of CD and guest molecules attracts intense interest in fabricating regular supramolecular amphiphilic structures with special properties, scilicet, vesicles bricks, as well as sheet-like structures.[20–23] For example, Ravoo and co-workers used self-assembled CD supramolecular vesicle as templates to fabricate hydrophilic polymer nanocontainers by using the host–guest interaction of adamantane and β-cyclodextrin.[24] Yan and co-workers reported a smart bacteria-like vesicles with photoswitchable cilia fabricated from CD–guest interactions, which can be used as smart nanocarriers.[25] Furthermore, the macroscopic gel self-assembly through host–guest interactions endows the function of molecular recognition, providing the application of controlled switchable structures sensitive to photo, redox, solvent, pH, and temperature.[26–30] To the best of our knowledge, however, researches on the self-assembly of CD derivatives constructed by designing specific functionalized monomolecular CD molecules are limited.

Recently, our group has studied hyperbranched polymers functionlized by anthracene, which could self-assemble into microspheres, core–shell microspheres, nanorods, as well as nanosheets.[31–34] The introduction of anthracene moieties makes the assembly system more hydrophobic, meanwhile anthracene can undergo photo-dimerization to stabilize the self-assemblies. On the basis of these work, we here designed a novel kind of amphiphilic macromolecule based on anthracene-functionalized β-cyclodextrin (CD-AN) prepared through

Cu(I)-catalyzed azide–alkyne click reactions, which could further self-assemble into spherical particles in water through multi-micelle aggregation (MMA) (Scheme 1). We traced the aggregation of CD-AN and the formation of aggregates from primary core–shell micelles to secondary aggregates, and especially observed the morphology of primary assemblies. The photo-dimerization of anthracene moieties led to crosslink assembles, which assisted to observe the formation of nanomicelles during self-assembly, and confirm the MMA mechanism.

2. Experimental Section

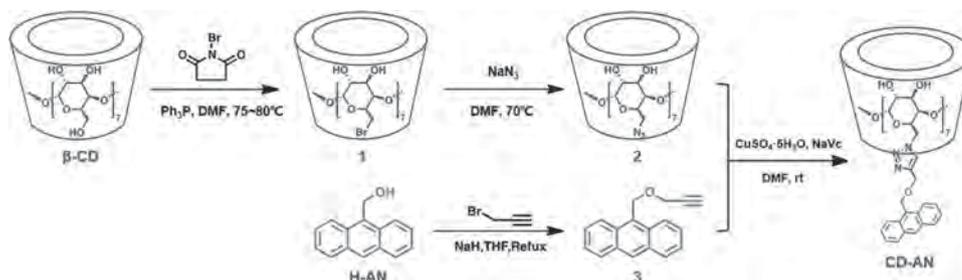### 2.1. Synthesis Process of CD-AN

See the Supporting Information.

2.2. Self-Assembly Method

Typically, the CD-AN self-assemblies were prepared in the mixture solvent. The CD-AN powder was first dissolved in dimethyl formamide (DMF) with the initial concentration of 5 mg mL^{-1}. Then mili-Q water as well as other solvents were added dropwise to the CD-AN solution with stirring at an addition rate of 83.33 μL min^{-1} which was controlled by an injection pump at 30 °C to obtain different final concentration of self-assemblies.

3. Results and Discussion

3.1. Synthesis and Characterization of CD-AN

Scheme 2 illustrates the whole strategy for the synthesis of CD-AN via a Cu(I)-catalyzed azide–alkyne click reaction in

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■ *Scheme 2.* Strategy for the synthesis of CD-AN via the azide–alkyne click reaction.

which β-cyclodextrin can be functionalized by anthracene with propargyl groups to form amphiphilic compounds. The reactant of heptakis (6-deoxy-6-azido)-β-cyclodextrin (**2**) with 1 equiv of 9-anthracenemethyl propargyl ether (**3**) was dissolved in DMF at room temperature with sodium ascorbate and $CuSO_4 \cdot 5H_2O$ as the catalyst, to afford CD-AN.

The ^1H NMR characterization of amphiphilic macromolecule CD-AN has been provided in Figure 1, which reveals the complete reaction between azido and alkynyl. The peak 7 is assigned to the protons on triazole corresponding to the azide–alkyne click reaction. ^{13}C NMR spectra and matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) data further supported the successful synthesis of the targeted CD-AN (Figures S2c and S4, Supporting Information). The structures of the obtained compounds were characterized by ^1H and ^{13}C NMR, Fourier transform infrared (FT-IR) and mass spectrometry (MS) measurements (see details in the Supporting Information).

3.2. Self-Assembly of CD-AN in Water

Self-assembly CD-AN was carried out through a gradually water adding process to DMF solution of CD-AN. With the increasing volume percentage ratio of water in DMF solution, namely, from 0% to 80%, solution opacity as well as Tyndall scattering increased (Figure S5, Supporting Information). As water is a kind of preferable solvent

for β-cyclodextrin and poor solvent for anthracene, the microphase separations in amphiphilic CD-AN tend to reinforce when the ratios of water increased, resulting in an apparent turbid of the solution and self-assembly of CD-AN. For the self-assemblies, the color of fluorescence emission changed obviously from hyaline to light yellow-green along with the addition of water, which could be seen under UV-light (Figure 2a). This was further confirmed by the steady-state fluorescence spectra (Figure 2b). Upon adding water, the system exhibited characteristics of an aggregation-induced emission enhancement (AIEE) as well as aggregation-induced emission (AIE).[35] Specifically, the fluorescence of CD-AN powder dissolved in DMF was feeble due to the well-known concentration-quenching effect of aromatic compounds because of the free molecular torsional motion.[36] The hydrophobic anthracene moieties aggregated during the process of self-assembly, which to a
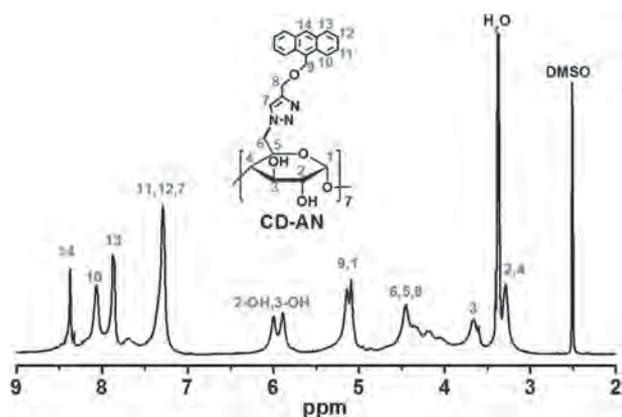
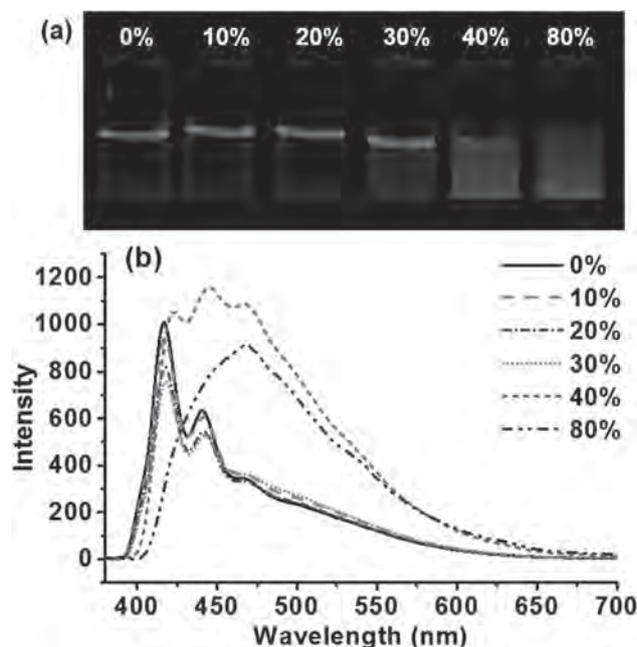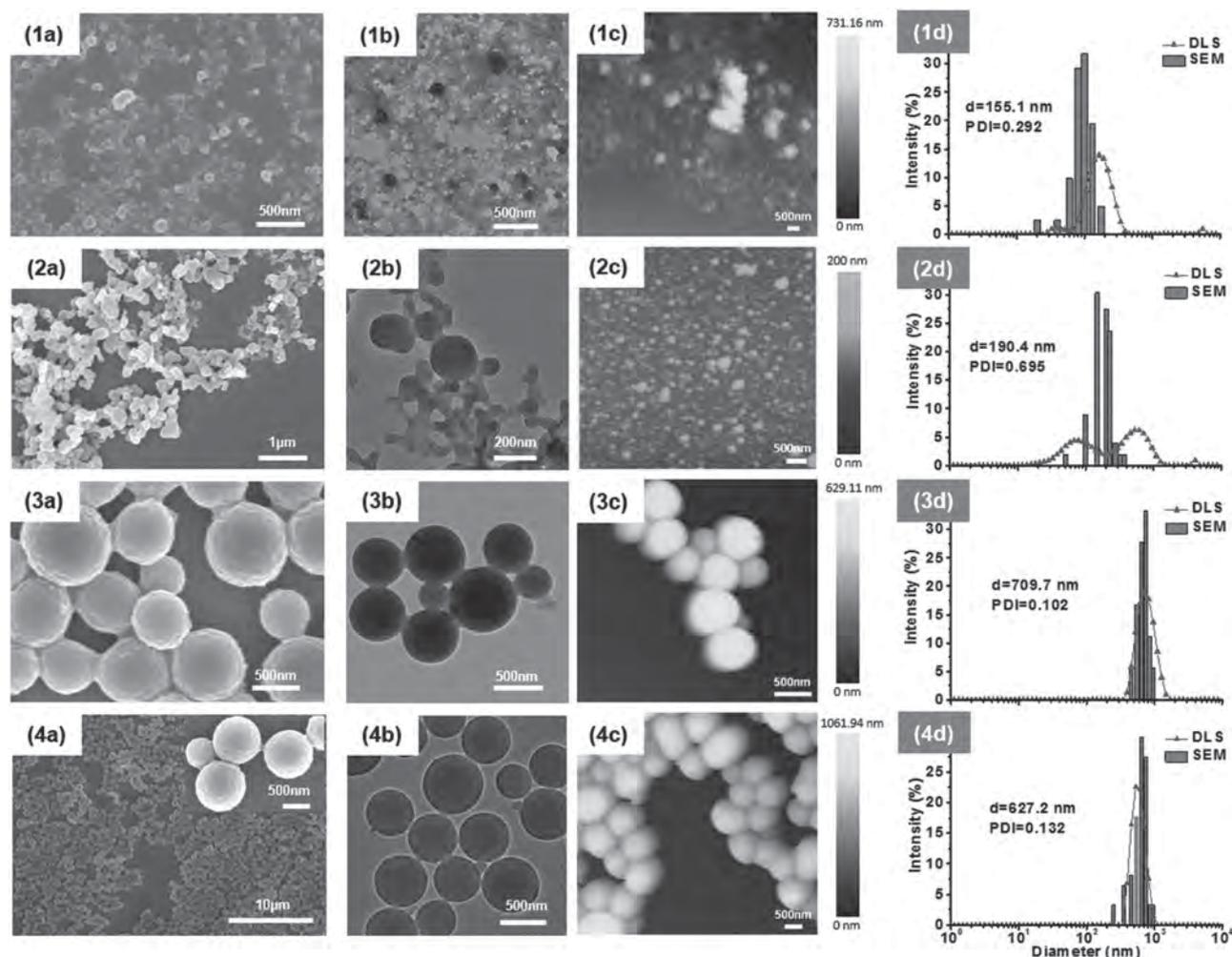■ *Figure 1.* ^1H NMR spectra for CD-AN in DMSO-d_6.

■ *Figure 2.* a) Photograph of self-assembled CD-AN in mixed solvents of H_2O/DMF with the volume ratio of water in mixture: 0%, 10%, 20%, 30%, 40%, and 80% under 365 nm UV-light illumination in the dark; b) steady-state fluorescence spectra of CD-AN in mixed solvents, excitation wavelength is 370 nm.

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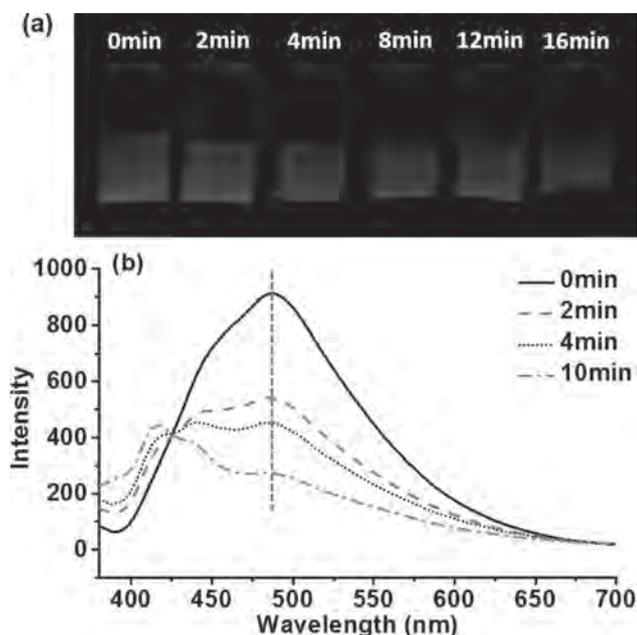
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Figure 3. Morphology and size distribution of self-assembled CD-AN: a) SEM images; b) TEM images; c) AFM images; d) size-distribution determined by DLS and SEM. Key: water content of 10% (line 1), 20% (line 2), 40% (line 3), and 80% (line 4).

certain extent restricted the dominant nonradiative relaxation, leading to a fluorescence enhancement (namely, AIE). At the same time, an emission peak at ≈467 nm became extremely conspicuous according to the fluorescence spectra when water content raised upon 40%, which suggested the strong π–π interaction among anthracene during the formation of CD-AN self-assemblies (AIEE).

To investigate the morphology of CD-AN self-assemblies, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) were performed on air-dried samples (Figure 3a–c). The particle sizes statistically analyzed from SEM were mainly in accord with the average hydrodynamic diameter (D_h) monitored by dynamic light scattering (DLS) (Figure 3d). Overall, self-assemble samples with different water content were observed to form spherical particles, which were noted to have increasing sizes when the volume percentage ratio of water increased. When the water content was below a critical point, the amphiphilic macromolecules first aggregated in primary micelles which had hydrophilic cyclodextrin sections in the periphery and hydrophobic anthracene parts interiorly because of the microphase separation. With the addition of water, the size of the self-assemblies was traced measured by DLS, which basically matched with the size distribution statistically analyzed in SEM images (Figure 3d). Interestingly, AFM images (Figure 3c) of the dried particles suggest that the self-assemblies possess extraordinarily rigid structure. This might be ascribed to the strong interaction among cyclodextrin molecules, which was regarded as crystalline aggregation further authenticated by X-ray diffraction measurements (Figure S6, Supporting Information). The strong major diffraction peaks at ≈9.0° and ≈12.6° reveal the cage-type cyclodextrin structures, which become no longer noticeable after being self-assembled.[37]

The resulting assembly of CD-AN was further cross-linked through photo-dimerization of anthracene using

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Figure 4. Process for photo-crosslinking of self-assembled CD-AN with the water content of 80%: a) photograph under 365 nm UV-light illumination in the dark and b) steady-state fluorescence spectra of CD-AN (excitation wavelength is 370 nm).

365 nm ultraviolet LED lamp (Uvata, China) with intensity about 8.4 mW cm^{-2}, which was traced by UV–vis spectra (Figure S7, Supporting Information). After irradiation of 365 nm light for 8 min, about 90% anthracene moieties were photo-dimerized. Irradiation time of 16 min was used to ensure the assembly of CD-AN well cross-linked. Figure 4a presents the fluorescent pictures of cross-linked assemblies, and the light yellow-green fluorescence of CD-AN assembly turned pale cyan after cross-linking.

With increasing degree of crosslinking, the fluorescent emission intensity at ≈467 nm decreased obviously (Figure 4b), which basically implied that the aggregates of anthracene get close and well-ordered.

While CD-AN can self-assemble into stable, uniform spherical particles in water (Figure 5b) via the process of MMA, the primary aggregation structure deserves demonstration. It is remarkable to see that the multi-micelle aggregates could disaggregate into primary micelles even if the whole system has been under photo-crosslinking, which is difficult to observe in other common crosslinking cases. Owing to the perfect ranging of CD-AN molecules designed on purpose in the well-defined primary core–shell structure, namely, the total ranging of anthracene interiorly, when anthracene groups in the core undergo photo-dimerization under 365 nm UV illumination, the photo-crosslinking process occurs only intra-nanoparticle instead of inter-nanoparticles, thus resulting in the fixation of the primary aggregation units. Once changing the solution medium conditions, for example, altering solvents or adjusting pH value, the cross-linked self-assemblies disaggregate into smaller micelle units that are considered to be the fundamental structure of the aggregates, rather than completely disassemble into molecules (Figure 5a). SEM images reveal that the size of the self-assemblies decreases from around 700 nm to less than 100 nm as soon as the cross-linked samples are dissolved in DMF or THF, while TEM and AFM images also confirm the primary spherical aggregates with the diameter of several tens nm (Figure 5c,e,f). Similarly in the case of changing pH value of the assembly solution, the cross-linked CD-AN could disaggregate into primary micelles in acidic condition measured by DLS (Figure 5d, black curve) and reversibly aggregate into large micelles
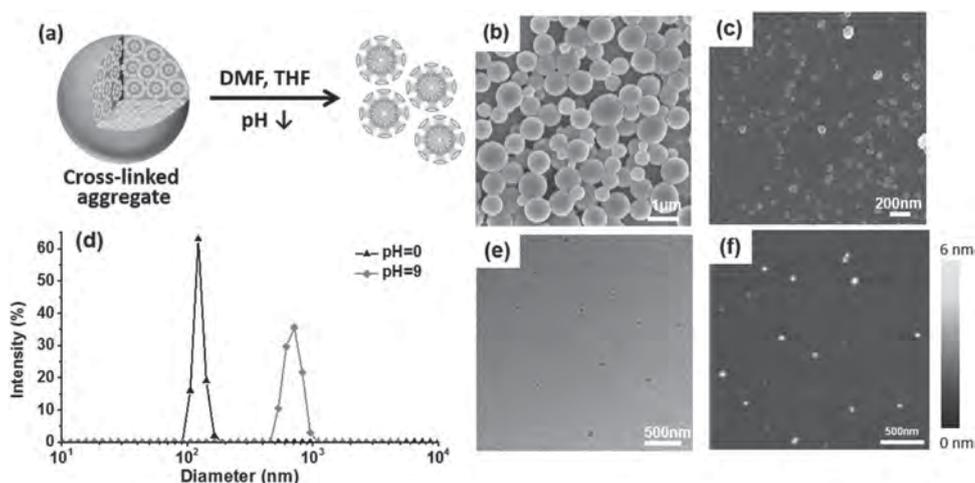
Figure 5. a) Schematic process for disaggregation in organic solvents or with the decrease of pH; b) SEM image of self-assembled CD-AN in water; c) SEM, e) TEM, and f) AFM images of disassembled CD-AN after UV crosslinking before dissolved in THF or DMF; d) DLS for tracing the change of intensity-average size distribution of CD-AN assemblies at different pH.

when pH was turned back to neutral or alkalinity (Figure 5d, red curve).

3.3. Self-Assembly of CD-AN in Other Solvents

To further confirm the special core–shell structure and the MMA mechanism, self-assemblies of CD-AN molecules in organic solvents were carried out. As shown in Figure S8a (Supporting Information), since CD-AN molecule rigidly distinguishes the hydrophilic and hydrophobic parts, when solvents that are preferable solvent for anthracene and poor solvent for β-cyclodextrin are added, the microphase separations will turn over, thereby resulting in the reverse assembly, which would not disaggregate in THF or DMF after photo-crosslinking just like other common crosslinking microparticles. CD-AN could self-assemble into primary spherical micelles with cyclodextrin sections in the interior core and anthracene parts on the peripheral shell, and then secondary aggregated into particles with less regularity and strong aggregation effect in both toluene and ethanol because of the strong π–π interaction between anthracene molecules (Figures S8b,c and S9, Supporting Information). Different from self-assembly in water, the photo-dimerization process in these cases proceeded both intra-nanoparticle and inter-nanoparticles, resulting in the crosslinking of both anthracene shell of primary micelle and entire particles. Therefore, the microparticles could not disaggregate when changing the solution conditions (Figure S8d, Supporting Information).

4. Conclusions

In summary, we demonstrated that amphiphilic CD-AN can self-assemble into strong, regular spherical particles in water via the process of MMA from primary core–shell micelles to secondary aggregates, followed by crosslinking via photo-dimerization of anthracene. Particularly, when the solution condition is adjusted, the cross-linked self-assemblies can readily disaggregate into primary aggregate units on account of the rigid and complete packing of anthracene in the core, which distinguishes from other common non-disassembled crosslinking systems. In solvents on the contrary, the amphiphiles can assemble into reverse aggregates but cannot disaggregate after photo-crosslinking. In general, the reversible MMA processes during self-assembly can only be observed when the primary aggregate units are fixed, while the core–shell CD-AN primary nanoparticles are capable of being cross-linked intra-nanoparticle exactly, therefore providing a new way of oriented design and self-assembly of functionalized amphiphilic macromolecules in the process of MMA.

Supporting Information

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