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Recent Progress in Fluorescent Vesicles with Aggregation-induced Emission

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Abstract Fluorescent vesicles have recently attracted increasing attention because of their potential applications in bioimaging, diagnostics, and theranostics, for example, *in vivo* study of the delivery and the distribution of active substances. However, fluorescent vesicles containing conventional organic dyes often suffer from the problem of aggregation-caused quenching (ACQ) of fluorescence. Fluorescent vesicles working with aggregation-induced emission (AIE) offer an extraordinary tool to tackle the ACQ issue, showing advantages such as high emission efficiency, superior photophysical stability, low background interference, and high sensitivity. AIE fluorescent vesicles represent a new type of fluorescent and functional nanomaterials. In this review, we summarize the recent advances in the development of AIE fluorescent vesicles. The review is organized according to the chemical structures and architectures of the amphiphilic molecules that constitute the AIE vesicles, *i.e.*, small-molecule amphiphiles, amphiphilic polymers, and amphiphilic supramolecules and supramacromolecules. The studies on the applications of these AIE vesicles as stimuli-responsive vesicles, fluorescence-guided drug release carriers, cell imaging tools, and fluorescent materials based on fluorescence resonance energy transfer (FRET) are also discussed.

Keywords Vesicles; Polymersomes; Aggregation-induced emission; Small-molecule amphiphiles; Amphiphilic polymers; Amphiphilic supramolecules; Amphiphilic supramacromolecules

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INTRODUCTION

Amphiphilic molecules composed of both hydrophilic and hydrophobic parts can self-assemble in aqueous media to form a wide variety of morphologies, such as micelles, vesicles, nanofibers, nanotubes, and multi-geometry nanoparticles.^[1–5] Among these nanostructures, vesicles have attracted considerable research interests. Vesicles are basically a nanostructure with spherical shell in which an aqueous chamber is enclosed by a membrane made from amphiphiles. The hydrophobic part of the amphiphiles tends to pack together to avoid the direct exposure to water, whereas hydrophilic part preferentially faces the inner and outer aqueous media.^[2,6] Such a nanostructure is expected to encapsulate not only hydrophobic agents in the membrane, but also hydrophilic ones in the inner aqueous compartment.^[7,8] More importantly, the properties of vesicles can be extensively tailored by taking advantage of the synthetic nature of the amphiphilic molecules.^[9] For example, biochemically active functional groups or specific cell targeting groups can be incorporated at the hydrophilic surfaces of vesicles for targeted delivery and imaging appli-

cations.^[10–12] On the other hand, the hydrophobic domains of vesicles can be engineered with segments that respond to pH change, temperature variation, light illumination, redox potential change, magnetic field, and ultrasound, *etc.*, in order to achieve controlled release of encapsulated substances.^[12–17] In addition, robust vesicles with high stability, low permeability, and low fluidity are accessible by crosslinking of the membrane or appropriate designing of the molecular architecture (*e.g.*, using amphiphiles with high molecular weight).^[6,18,19] Due to these beneficial features, small molecule vesicles and polymer vesicles (polymersomes) have been studied intensively for the application of drug delivery with the aim of reducing drug toxicity, avoiding the decomposition of active molecules, and controlling the release of entrapped agents in time and in space.

Fluorescent probes are one of the most powerful tools for real-time, non-invasive, and on-site visualization of morphological details in cells and of biological processes in cells and organisms.^[20–25] Fluorescent nanostructures provide many advantages over discrete molecular fluorescent probes in terms of sensitivity, photostability, and cellular retention.^[26] Over the past decades, a growing interest has been shown in the design of different fluorescent vesicles because of their multi-targeting abilities and unique encapsulation capabilities for hydrophilic and hydrophobic drugs as well as their fluorescence features. Fluorescent vesicles can find ap-

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plications in cell/tissue imaging and *in vivo* study of drug distribution and delivery, *etc.*^[26–30] Currently, organic dyes are the most commonly used fluorescent probes due to their color diversity, rich chemical structures, and functionalities.^[26] However, ubiquitous aggregation-caused quenching (ACQ) phenomenon is not always easy to avoid for conventional organic dyes.^[31] They often show bright emission in solutions, but have their fluorescence largely weakened or even quenched in high concentrations or aggregated state. Very interestingly and fortunately, a new category of fluorogens with aggregation-induced emission (AIEgens), which presents the opposite fluorescence phenomenon to ACQ, has been developed by Tang's group.^[32,33] AIEgens are non-emissive in solution, but highly fluorescent when they are aggregated. Mechanistic analyses of the AIE processes indicate the restriction of intramolecular motions (RIM) is one of the main mechanisms of AIE effects.^[33,34] Vesicles could offer this restriction in a controlled way upon self-assembling AIEgen-containing amphiphilic molecules into nanosized bilayers. The combination of AIEgens and vesicles will provide innovative approaches for bioimaging, diagnostics, and theranostics.

In this review, we summarize the recent advances in the development of AIE fluorescent vesicles. These AIE vesicles include vesicles made of small-molecule amphiphiles, amphiphilic polymers, and amphiphilic supramolecules/supramacromolecules. Therefore, the review will be organized mainly in three parts: small-molecule AIE vesicles, AIE polymersomes, and AIE vesicles made of supramolecules/supramacromolecules. Their applications as stimuli-respon-

sive vesicles, fluorescence-guided drug release carriers, cell imaging agents, and fluorescent materials based on fluorescence resonance energy transfer (FRET) will also be described. Finally, the limitations and future perspectives of AIE vesicles will be discussed.

AIE VESICLES FORMED BY SMALL-MOLECULE AMPHIPHILES

As an analogue to phospholipids, synthetic small-molecular amphiphiles containing AIEgens can self-assemble into AIE vesicles when their hydrophilic/hydrophobic ratio is in the suitable range. In the last decade, small-molecule AIE amphiphiles with hydrophilic moieties, like ionic groups, peptides, and oligo(ethylene glycol), have often been used as tools for optical sensing and imaging.^[22,35] However, the studies of direct formation of AIE vesicles with small-molecule AIE amphiphiles are still limited in number. We discuss here two examples: one with ammonium ion as hydrophilic group, and another with bile acid as hydrophilic part.

Zhang *et al.* reported AIE vesicles based on a cationic amphiphile.^[36] As shown in Fig. 1(a), the amphiphile **1** consisted of a hydrophilic ammonium ion and the hydrophobic TPE derivative. Moreover, two acrylate groups were connected to the hydrophilic head for later crosslinking. The amphiphile **1** could spontaneously self-assemble into vesicles in aqueous solution. The average diameter of the self-assembled vesicles (TPE-Vs) was ~106 nm as measured by dynamic light scattering (DLS) (Fig. 1b). The vesicles disper-

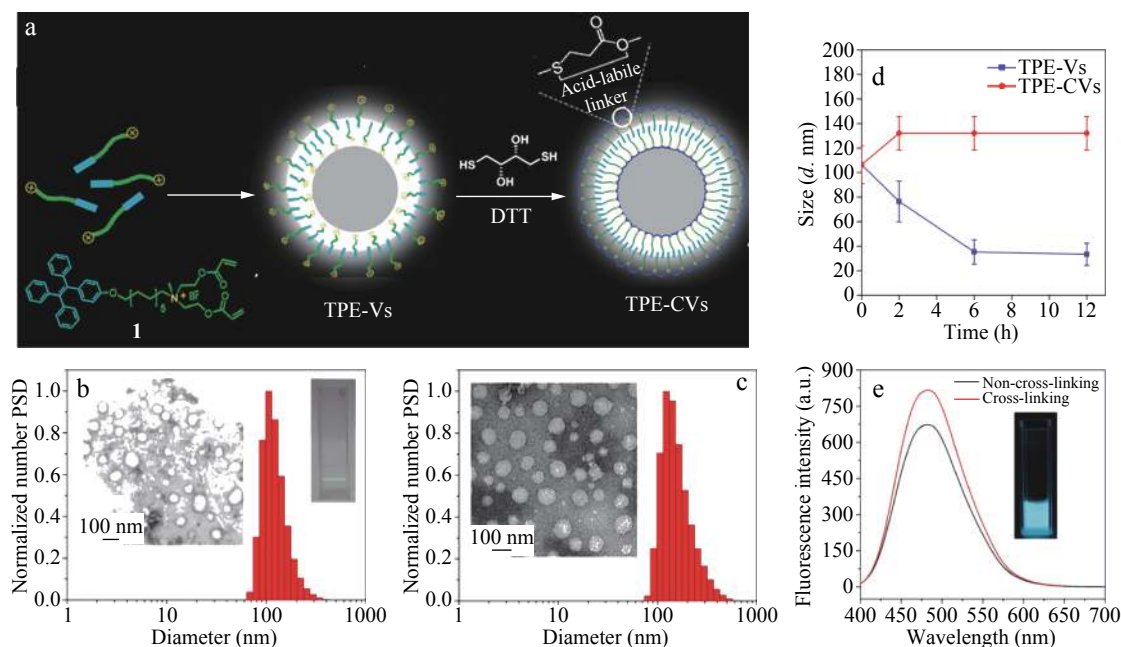


Fig. 1 (a) Schematic illustration of preparation of vesicles with amphiphile **1**; (b) Size distribution and TEM image of vesicles TPE-Vs formed by amphiphiles **1** in aqueous solution. The right inset image shows the Tyndall effect of the vesicles solution under a laser of 532 nm; (c) Size distribution and TEM image of cross-linked vesicles TPE-CVs; (d) Particle sizes of TPE-Vs and TPE-CVs after incubation with 10% FBS at 37 °C over time ($[1] = 0.25 \text{ mmol} \cdot \text{L}^{-1}$); (e) The fluorescence spectra of vesicles before and after cross-linking, $\lambda_{\text{ex}} = 373 \text{ nm}$. Inset photograph of TPE-CVs solution was taken under a handheld UV lamp (365 nm). (Reproduced with permission from Ref. [36]; Copyright (2018) American Chemical Society)

sion gave strong fluorescence at ~ 480 nm with the excitation wavelength of 373 nm (Fig. 1e). Further, the cross-linker dithiothreitol (DTT) was added to the vesicle's dispersion. Its $-SH$ groups reacted with the alkenyl groups at the surface of TPE-Vs by thiol-acrylate Michael addition, leading to the cross-linked AIE vesicles (TPE-CVs) (Fig. 1a). The cross-linked TPE-CVs could maintain their sizes when treated with 10% FBS at 37 °C until 12 h of incubation, in contrast to the case of non-crosslinked TPE-Vs whose sizes decreased dramatically with the incubation time (Fig. 1d). The results of DLS and TEM indicated a slight increase in average diameter of TPE-CVs compared to that before cross-linking (Fig. 1c). More interestingly, the fluorescence intensity increased significantly from TPE-Vs to crosslinked TPE-CVs, even though the emission band position did not change after cross-linking (Fig. 1e). The augmentation of the fluorescence intensity was explained by the enhanced restriction of the intramolecular rotation of TPE moieties upon cross-linking. In addition, the cross-linked vesicles showed a higher quantum yield ($\Phi_f = 17.7\%$) than that of TPE-Vs ($\Phi_f = 13.2\%$).

By taking advantage of FRET from the aggregated TPE moieties to hydrophilic rhodamine B (RhB) acceptors encapsulated inside the aqueous compartment of TPE-CVs, Zhang *et al.* constructed multicolor emission vesicles TPE-CVs@RhB by altering the amount of entrapped RhB. A gradual decrease of the fluorescence intensity for TPE moiety ($\lambda_{em} = 475$ nm) was observed with the increasing amounts of entrapped RhB when excitation wavelength was fixed at 373 nm (Fig. 2a). By contrast, the emission of acceptor RhB at $\lambda_{em} = 578$ nm tended to increase steadily (Fig. 2a). Fig. 2(b) shows the map of emission of TPE-CVs@RhB on the CIE 1931 chromaticity diagram by changing the concentration of RhB. TPE-CVs@RhB could change its color from blue to white and then to orange-red with the increasing RhB content. The photographs of the TPE-CVs@RhB dispersion in aqueous solution with different amounts of RhB under UV light (365 nm) are consistent with the result of CIE diagram (Fig. 2c). The vesicle dispersion exhibited blue emission in

the absence of RhB, white emission in the presence of 0.36 mol% of RhB, and orange-red when further increasing the RhB concentration to 2.4 mol%.

In the second example of small-molecule AIE vesicles, the hydrophilic group is not ionic, but the hydroxyl groups in bile acids. Bile acids are a class of amphiphilic molecules which have a nonplanar steroidal skeleton with a convex hydrophobic surface and a concave hydrophilic face with $-OH$ groups.^[37] Li *et al.* reported bile acids-based AIE vesicles with amphiphilic binding pockets in the membrane (Fig. 3) by exploring the synergetic self-assembly of both TPE and bile acid building blocks.^[38] By synthesizing a series of TPE-bile acid conjugates 1–5 (Fig. 3a), they demonstrated that only compounds 1, 2, 3, and 5 with at least two hydroxyl groups in the bile acid unit could self-assemble into vesicular nanostructures with average diameter of 200 nm in the co-solvent systems (water/acetone or water/methanol). They suggested that the hydrophilic-hydrophobic balance of bile acid units and the hydrogen-bonding probability have critical influence on the morphologies of the self-assemblies. They pointed out that *E* isomer (compound 2) could self-assemble into monolayer vesicles, while *Z* isomer (compound 5) could form bilayer vesicular structures (Figs. 3b–3d).

More interestingly, the amphiphilic binding pockets in the membrane of AIE vesicles as shown in Fig. 3(b) provided a unique opportunity for facile construction of FRET system with both hydrophobic and hydrophilic acceptors. Figs. 4(a) and 4(b) show the excellent overlap of fluorescence emission of vesicles (V2 prepared from compound 2) and absorption of acceptors of hydrophobic Nile red (NR) or hydrophilic RhB. By encapsulating NR into the hydrophobic binding sites of vesicular membrane, the original fluorescence emission of the vesicle at 462 nm gradually decreased, and a new emission band appeared at 605 nm initially, then shifted to 620 nm (Fig. 4c). An energy transfer efficiency of 52.4% was achieved. The similar FRET process could also be successfully realized by inserting hydrophilic RhB into the hydrophilic binding sites in the vesicular membrane

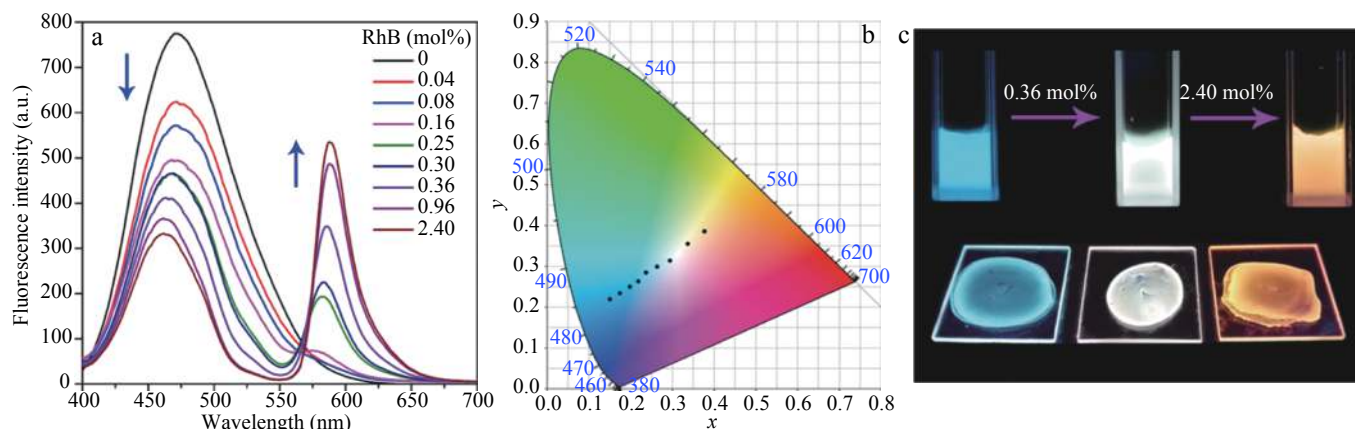


Fig. 2 (a) Fluorescence spectra of TPE-CVs@RhB with different amounts of RhB in water ($\lambda_{ex} = 373$ nm); (b) CIE chromaticity diagram showing luminescent color changes of TPE-CVs@RhB; (c) Photographs of the TPE-CVs@RhB dispersion in water with different concentrations of RhB (top) and the corresponding solid fluorescence microscopic images (bottom) under 365 nm UV light (Reproduced with permission from Ref. [36]; Copyright (2018) American Chemical Society)

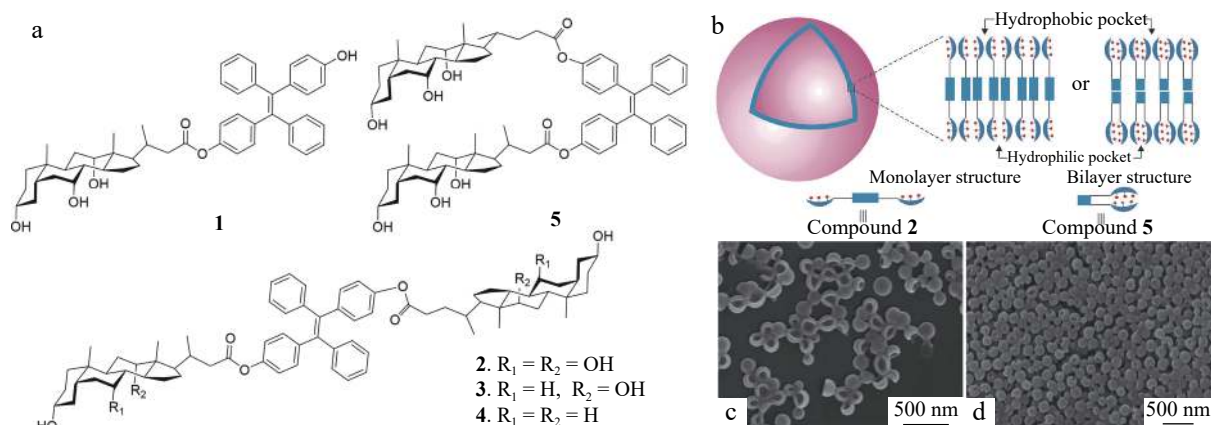


Fig. 3 (a) Chemical structure of TPE-bile acid conjugates 1–5; (b) Schematic illustration of the vesicles derived from compounds 2 and 5, which contain amphiphilic binding pockets; SEM images of the vesicles derived from (c) compound 2 at $f_w = 40\%$ and (d) compound 5 at $f_w = 50\%$ in water/acetone co-solvent system (Reproduced with permission from Ref. [38]; Copyright (2015) The Royal Society of Chemistry)

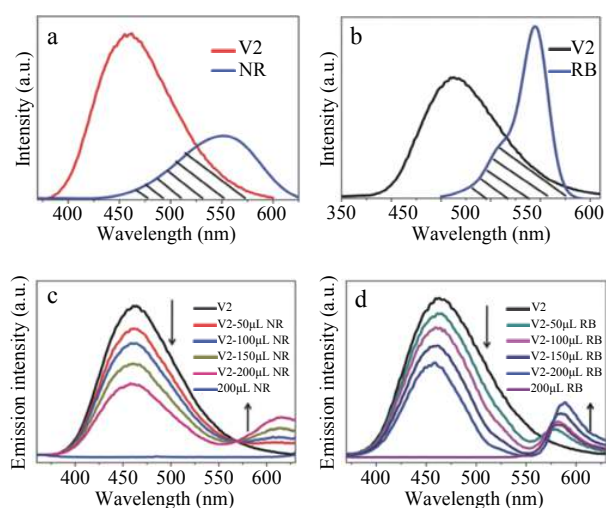


Fig. 4 Absorption and emission spectra of (a) the vesicle V2 and NR couple and (b) the vesicle V2 and RhB couple (RhB is abbreviated as RB in this figure); Fluorescence evolution of vesicle V2 on the encapsulation of (c) NR and (d) RhB; $I_{\text{ex}} = 325 \text{ nm}$ (Reproduced with permission from Ref. [38] Copyright (2015) The Royal Society of Chemistry)

(Fig. 4d), and an energy transfer efficiency of 41.8% was recorded.

AIE VESICLES FORMED BY AMPHIPHILIC POLYMERS

The synthetic AIE amphiphilic polymers that could form AIE polymer vesicles (AIE polymersomes) have similar amphiphilicity to that of AIE small-molecule amphiphiles, but show much higher molecular weights. Therefore, AIE polymersomes have many superior properties over small-molecule AIE vesicles, such as higher toughness and better stability.^[6,39] As a hydrophobic molecule, the AIE moiety is normally incorporated in the hydrophobic block of AIE amphiphilic polymers. Herein, we discuss several examples of AIE polymersomes according to the chemical structures and synthetic methods of the AIE amphiphilic polymers.

AIE Amphiphilic Block Copolymers Prepared by Post-functionalization

A simple strategy to generate amphiphilic AIE block copolymer is to post-functionalize AIEgens with one or several blocks of block copolymers.

Wu's group developed AIE amphiphilic diblock copolymers PEG-POSS-(TPE)₇ by attaching TPE groups to the multi-terminals of POSS block in the pre-copolymers PEG-POSS *via* Schiff base bonds (Fig. 5).^[40–42] In these copolymers, poly(ethylene glycol) chain serves as the hydrophilic block, and rigid cage-shaped POSS functionalized with TPE units as the hydrophobic block. Depending on the ratio of hydrophilic block, PEG-POSS-(TPE)₇ could self-assemble into polymersomes or polymer micelles by nanoprecipitation method in THF/water co-solvent system (Fig. 5a). PEG₃₅₀-POSS-(TPE)₇, with the short PEG chain ($M_n = 350$), self-assembled into polymersomes with diameter of 83 nm (Fig. 5b). Further increasing the PEG molecular weight to $M_n = 750$, polymersomes with large diameter of 350 nm could be formed.^[42] However, when the molecular weight of hydrophilic PEG extended to $M_n = 1900$, spherical micelles were obtained with the average size of 60 nm. The vesicle formed by PEG₃₅₀-POSS-(TPE)₇ showed bright cyan fluorescence when excited at 330 nm. The rotation of TPE moiety in the membrane of polymersomes was restricted severely, which activate the AIE process.

Moreover, the Schiff base bonds in the polymer structure provided the self-assembled AIE polymersomes with pH responsive property. These AIE polymersomes were stable under alkaline or physiological condition (pH 7.4) but readily cleavable under acidic or endosomal conditions (pH 5.0–6.0) of the tumor cells. As shown in Fig. 5(c), the fluorescence intensity of aggregated TPE dropped sharply to 14% of initial level after acid treatment for 24 h. When doxorubicin (DOX) was encapsulated inside these AIE polymersomes, limited release of DOX (< 30%) was observed at pH 7.4 within 24 h (Fig. 5d). By contrast, at pH 5.0, the encapsulated DOX released rapidly into the solution due to breakup of the vesicles in acid condition, with 70% released in the

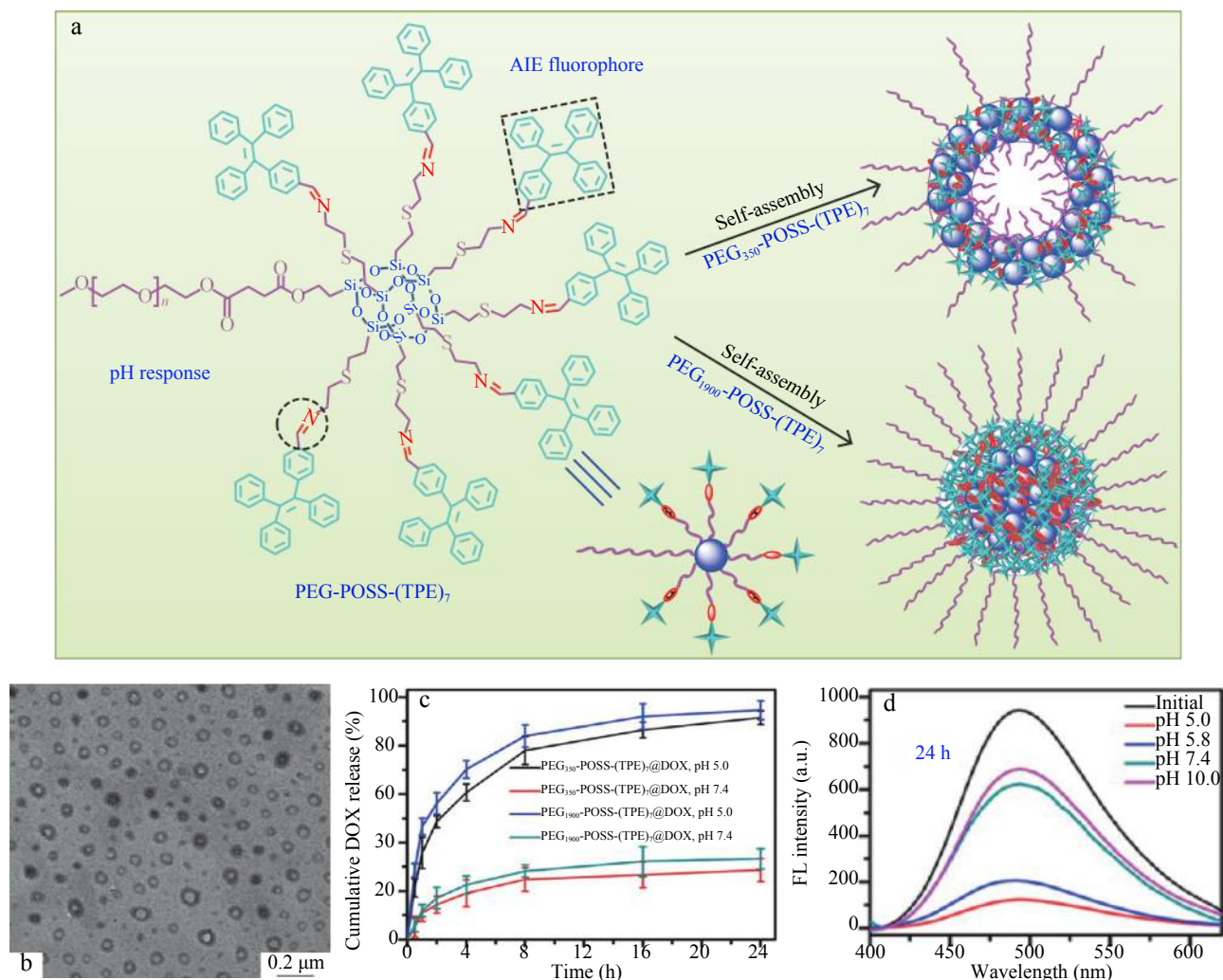


Fig. 5 (a) The chemical structure of PEG_n-POSS-(TPE)₇ and the schematic illustration of self-assembled polymersomes and polymeric micelles; (b) TEM image of PEG₃₅₀-POSS-(TPE)₇ polymersomes; (c) pH-triggered release of DOX from PEG₃₅₀-POSS-(TPE)₇@DOX (vesicles) and PEG₁₉₀₀-POSS-(TPE)₇@DOX (micelles) with various pH values (5.0 and 7.4) at 37 °C as a function of time; (d) Change of fluorescence intensity of PEG₃₅₀-POSS-(TPE)₇ vesicles in response to various pH values (5.0, 5.8, 7.4, and 10.0) for 24 h incubation (Reproduced with permission from Ref. [40]; Copyright (2018) American Chemical Society)

first 2 h and up to 95% in 24 h (Fig. 5d).

Li *et al.* have synthesized the first well-defined ABA triblock copolymer PVACB₁₃-PEG₄₅-PVACB₁₃ by reversible addition-fragmentation chain transfer (RAFT) radical polymerization (Fig. 6a).^[43] The hydrophobic blocks PVA in PVA₁₃-PEG₄₅-PVA₁₃ bear an azide group at each monomer unit, which was used to react with *N*-propargyl-carbazole by the click reaction to afford final amphiphilic triblock copolymer PVACB₁₃-PEG₄₅-PVACB₁₃ with carbazole fluorogens (Fig. 6a). Nanoprecipitation method in water with THF as cosolvent was applied to make polymer self-assemblies. Cup-shaped polymersomes were obtained with diameters ranging from 300 nm to 400 nm (Figs. 6b and 6c). The fluorescence intensity of polymersomes in water showed a significant augmentation compared to that of PVACB₁₃-PEG₄₅-PVACB₁₃ in chloroform solution (Fig. 6d). The restriction of intramolecular rotation motion of the carbazole groups in polymersomes membrane caused the AIE phenomenon.

AIE Amphiphilic Block Copolymers Prepared by Controlled Polymerization of AIE Monomers

Another strategy to prepare AIE amphiphilic block copolymers is to copolymerize the AIEgen-containing monomers by living or controlled polymerization, such as controlled radical polymerizations by reversible addition-fragmentation chain transfer (RAFT), ring-opening metathesis polymerization (ROMP), and ring-opening polymerization of cyclic carbonates, *etc.*

Chi *et al.* have synthesized by RAFT polymerization amphiphilic diblock copolymers, poly(vinyl tetraphenylethene)-block-poly(*N*-isopropylacrylamide) (PTPEE_m-*b*-PNIPAM_n) from TPE-substituted monomer vinyl tetraphenylethene and isopropylacrylamide (Fig. 7a).^[44] The PNIPAM_n block was a hydrophilic polymer at room temperature, while it became hydrophobic at temperature higher than its lower critical solution temperature (LCST) at around 32 °C. The amphiphilic copolymers PTPEE_m-*b*-PNIPAM_n self-assembled

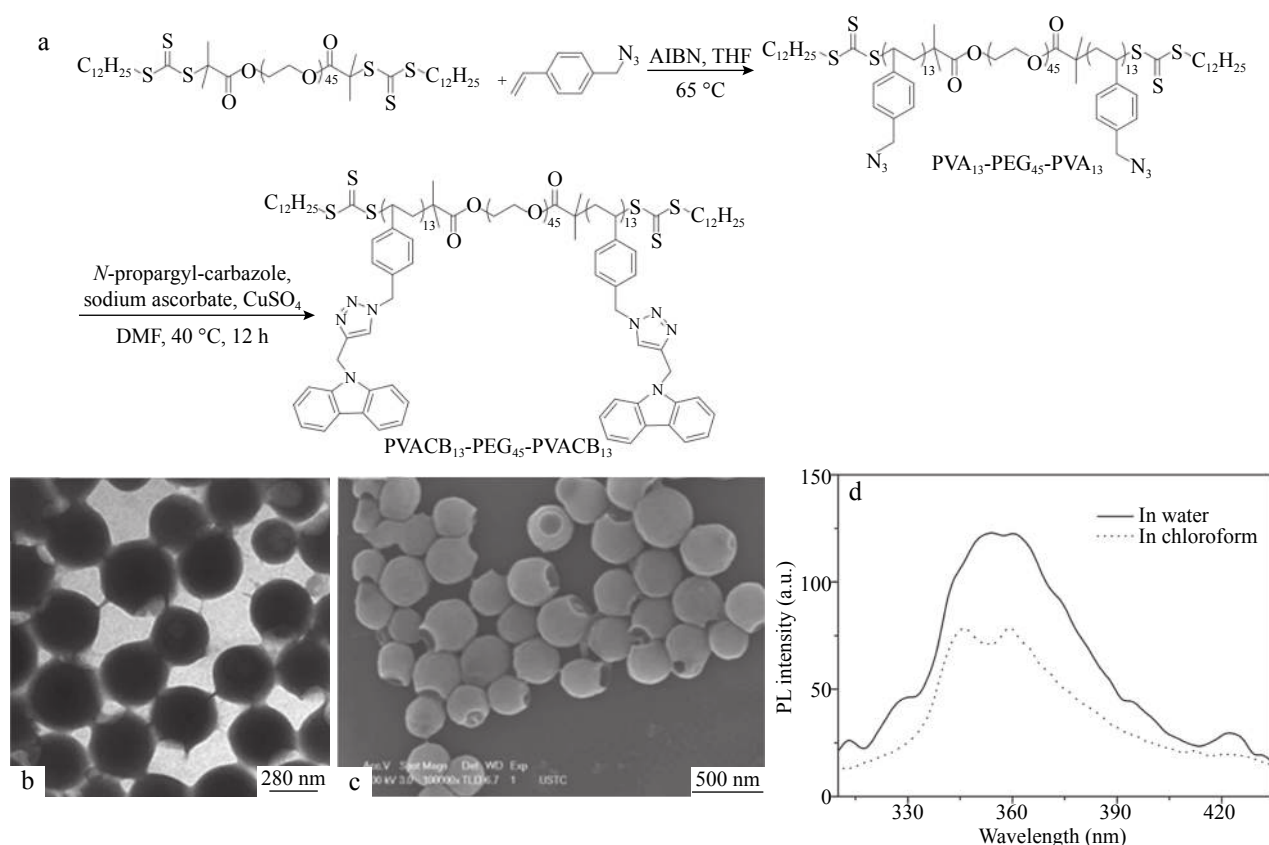


Fig. 6 (a) Synthetic route to PVACB₁₃-b-PEG₄₅-b-PVACB₁₃; (b) TEM image of cup-like polymersomes; (c) SEM image of cup-like polymersomes; (d) PL spectra of PVACB₁₃-PEG₄₅-PVACB₁₃ in chloroform and of polymersomes in water. Polymer concentration: 10⁻⁷ mol·L⁻¹; excitation wavelength: 295 nm. (Reproduced with permission from Ref. [43]; Copyright (2014) Elsevier Science)

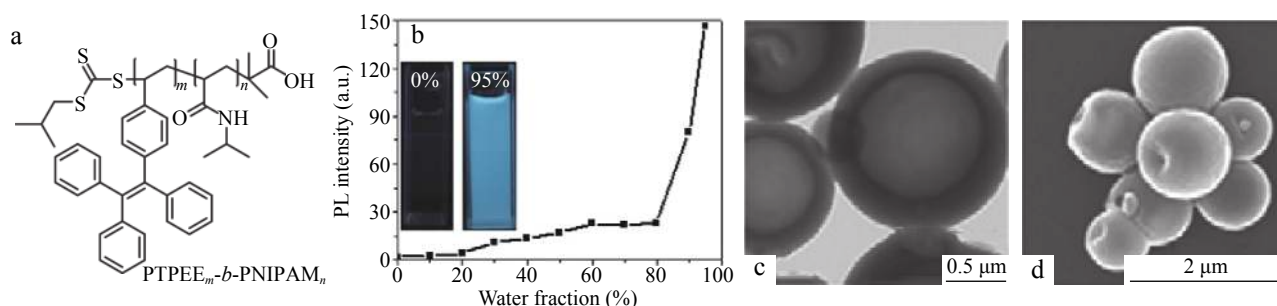


Fig. 7 (a) The structure of the copolymer PTPEE_m-b-PNIPAM_n; (b) AIE effect; (c) TEM and (d) SEM images of the polymersomes (Reproduced with permission from Ref. [44]; Copyright (2018) Elsevier Science)

into polymersomes in aqueous solution using nanoprecipitation method (Figs. 7c and 7d). The copolymers PTPEE_m-b-PNIPAM_n exhibited typical AIE property as shown in Fig. 7(b).

Zhang *et al.* prepared AIE amphiphilic block copolymers by ring-opening metathesis polymerization (ROMP).^[45,46] As shown in Fig. 8(a), a series of diblock copolymers, poly(M1)-b-poly(M2), with a fixed length of hydrophilic block poly(M2)₁₀ and different lengths of hydrophobic AIE block poly(M1), namely poly-(M1)₅₀-b-poly-(M2)₁₀, poly-(M1)₁₀₀-b-poly-(M2)₁₀, and poly-(M1)₃₀₀-b-poly-(M2)₁₀, were synthesized by ROMP using Grubbs catalyst G3. Different morphologies like spherical micelles, cylindrical micelles, and vesicles were formed by nanoprecipitation method

(Figs. 8c and 8d). Only poly(M1)₃₀₀-b-poly(M2)₁₀ with a high ratio of hydrophobic poly(M2) block self-assembled into polymersomes which emitted strong green fluorescence (see the inset image of Fig. 8d).

The same group has added supplemental functions in the AIE amphiphilic diblock copolymers (Fig. 9).^[46] They prepared poly(M1)-b-poly(M2-co-M3) whose hydrophobic block is a random copolymer made of bi(pentafluorophenyl ester)-substituted M2 and TPE-containing M3. The pentafluorophenyl ester groups are reactive sites for further functionalization. Polymersomes were formed only from poly-(M1)₁₀-b-poly(M2₁₀₀-co-M3₅) by nanoprecipitation method (Fig. 9b). However, spherical micelles and cylindrical micelles were obtained, respectively, from poly(M1)₁₀-b-

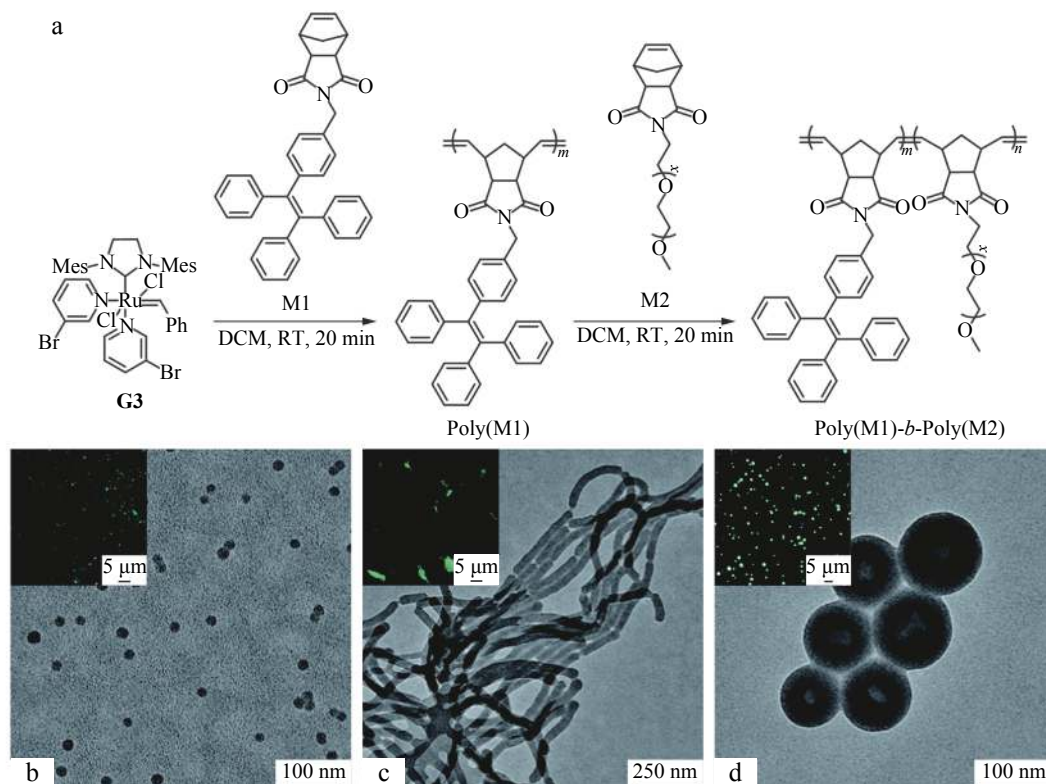


Fig. 8 (a) The synthetic route for AIE amphiphilic diblock copolymer of poly(M1)-*b*-poly(M2); (b) TEM image of the spherical micelles formed from poly(M1)₅₀-*b*-poly(M2)₁₀; (c) TEM image of the fibers formed from poly(M1)₁₀₀-*b*-poly(M2)₁₀; (d) TEM image of the polymersomes formed from poly(M1)₃₀₀-*b*-poly(M2)₁₀. Inserted picture in (b)–(d) is fluorescence microscope image of the aggregates. (Reproduced with permission from Ref. [45]; Copyright (2014) The Royal Society of Chemistry)

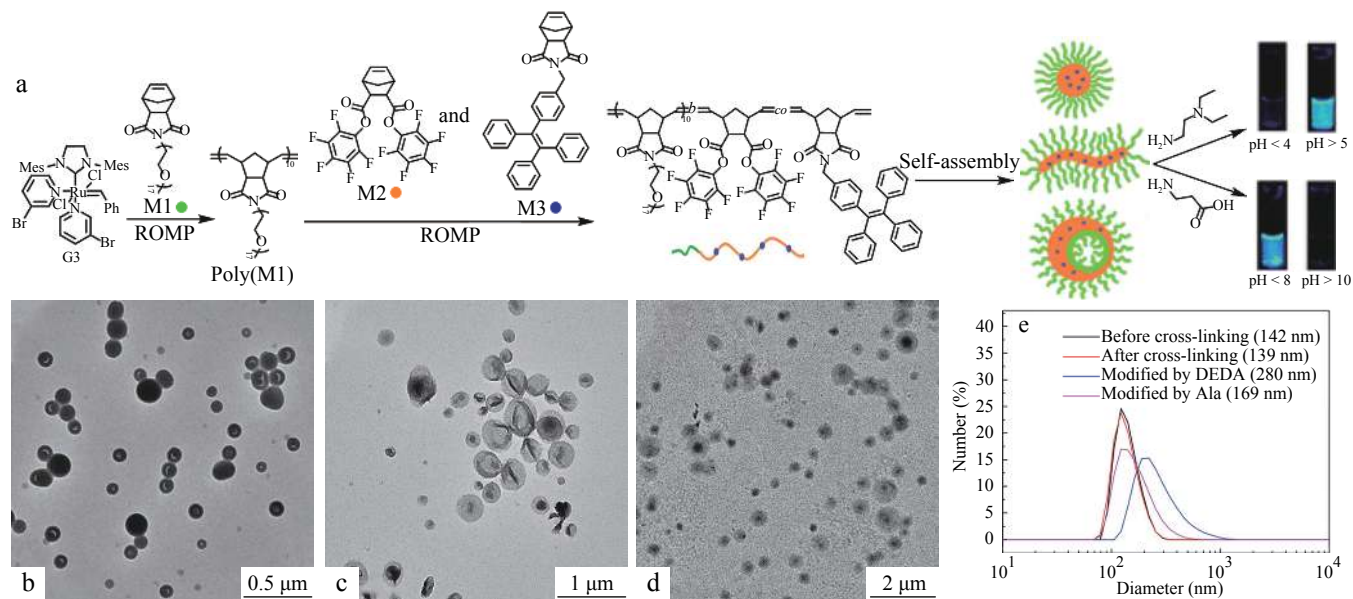


Fig. 9 (a) Preparation of poly(M1)-*b*-poly(M2-co-M3) block copolymers and the resultant stable polymeric nanostructure with pH-responsive and AIE properties; TEM images of (b) the polymersomes self-assembled from poly(M1)₁₀-*b*-poly(M2)₁₀₀-co-M3₅, (c) the DEDA post-functionalized polymersomes, and (d) the Ala post-functionalized vesicles; (e) DLS characterization of the polymersomes in water (Reproduced with permission from Ref. [46]; Copyright (2016) The Royal Society of Chemistry)

poly(M2₅₀-co-M3_{2.5}) and poly(M1)₁₀-*b*-poly(M2₈₀-co-M3₄), which had lower hydrophilic ratios. Additionally, the morphology of polymersomes could be fixed by slightly cross-linking their hydrophobic membrane, for which ethylene-

diamine (15 mol% of pentafluorophenyl ester groups) was added to react with pentafluorophenyl ester moieties in poly(M2-co-M3). Cross-linked polymersomes in water had the same average diameter as those before cross-linking

(Fig. 9e). The crosslinked polymersomes exhibited a large increase in size when dispersed in THF solution, which could be ascribed to the swelling of the crosslinked hydrophobic domain. This result demonstrated the success of cross-linking of polymersomes. They further post-functionalized the membrane of the slightly crosslinked polymersomes by introducing *N,N*-diethylethylenediamine (DEDA) or β -alanine (Ala) to react with the remaining activated pentafluorophenyl ester groups. The final polymersomes thus possess pH-responsive groups: diethylamino or carboxylic acid group. The morphology of the polymersomes was kept, but the diameter increased after the post-functionalization as shown in Figs. 9(c)–9(e). The presence of pH-responsive groups in the hydrophobic part of the membrane led to a tunable fluorescence of polymersomes *via* changing the pH as shown in Fig. 9(a). Ionization of the diethylamino group or of carboxylic acid group at appropriate pH rendered the increased hydrophilicity of the crosslinked microdomains in membrane. Consequently, the membrane was swollen by water and the restriction of intramolecular rotation of TPE was decreased. Eventually, DEDA-functionalized nanoparticles exhibited weak fluorescence at low pH, while Ala-functionalized nanoparticles showed low fluorescence at high pH.

Recently, our group synthesized a series of polycarbonate-based amphiphilic diblock copolymers using ROP with TPE-substituted cyclic trimethylene carbonate monomer, and studied minutely their self-assembling process into AIE polymersomes.^[47] Diblock copolymers $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_n$ with different lengths of AIE polycarbonate block ($n = 16, 13, 9$, and 7) were prepared using *m*PEG₄₅-OH as the macroinitiator and *N*-(3,5-trifluoromethyl)phenyl-*N'*-cyclohexylthiourea (TU)/1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (TU/DBU) as catalysts (Fig. 10a). Nanoprecipitation method in THF/water or dioxane/water system was then employed to afford polymersomes. We found that the diameter of polymersomes decreased when the hydrophobic block P(TPE-TMC) became shorter. After self-assembling in THF/

water, polymersomes of $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_{16}$ ($f_{\text{PEG}} = 17\%$) possessed an average diameter of 450 nm (Fig. 10b). Meanwhile, polymersomes of $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_{13}$ ($f_{\text{PEG}} = 21\%$) had an average diameter of 250 nm (Fig. 10c). $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_9$ ($f_{\text{PEG}} = 27\%$) and $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_7$ ($f_{\text{PEG}} = 33\%$) formed rather small polymersomes with average sizes of 90 and 70 nm, respectively (Figs. 10d and 10e). Thus, we could control the size of polymersomes by changing the length of hydrophobic block P(TPE-TMC). This could also be achieved by changing the co-solvent, *e.g.*, large vesicles of $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_{13}$ ($f_{\text{PEG}} = 21\%$) with an average diameter of 650 nm were obtained after self-assembling in dioxane/water. The size-control of nanoparticles is of importance to potential applications because their size could affect their cellular uptake.

The morphological evolution and the fluorescence emission during the whole self-assembling process were then examined carefully by nanoprecipitation (Fig. 11). We found two main stages in the evolution of fluorescence intensity: a slow increase at the water contents $f_w < 60\%$ followed by an abrupt increase of fluorescence intensity at $f_w > 60\%$ in THF/water system (Fig. 11). The first stage of fluorescence increase could be explained by the polymersome formation (Figs. 11c and 11d). The non-water-soluble P(TPE-TMC) block self-assembled into the core of membrane and the intramolecular motion of TPE moieties was restricted. Nevertheless, due to the presence of solvent (THF or dioxane) in the membrane, this restriction of motion was limited, and the fluorescence intensity was moderate. After the water content reached 60% (in THF/water), the sudden increase of fluorescence intensity upon further water addition could be caused by the removal of the organic solvent from the membrane. The concentration gradient of the organic solvent from inside to outside the membrane resulted in the release of THF by osmotic pressure. Consequently, the AIEgens, *i.e.* TPE moieties, aggregated effectively. As there were few solvent molecules to “lubricate” the intramolecular motion, the photoluminescence was activated efficiently to give high fluor-

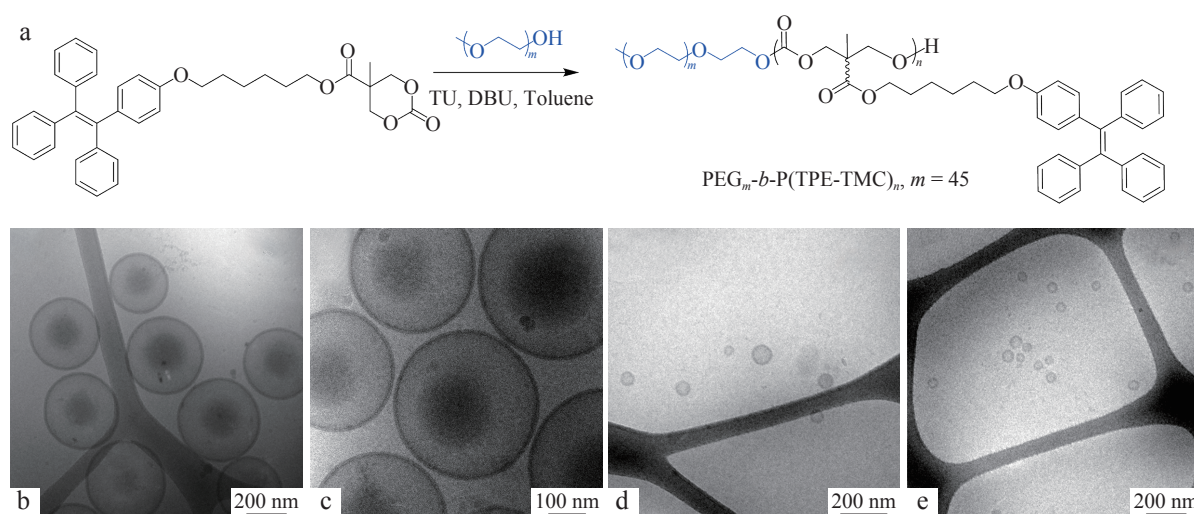


Fig. 10 (a) The synthetic route of polymer $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_n$; TEM images of (b) polymersomes formed from $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_{16}$, (c) polymersomes formed from $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_{13}$, (d) vesicles formed by $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_9$, and (e) polymersomes formed by $\text{PEG}_{45}\text{-}b\text{-P(TPE-TMC)}_7$ (Reproduced with permission from Ref. [47] Copyright (2018) American Chemical Society)

escence intensity (Fig. 11e). The turning point between these two stages appeared earlier in dioxane/water (50%) than that in THF/water (60%), since THF is a better solvent for P(TPE-TMC) than dioxane. Both polymersomes in pure water after dialysis against water exhibited very strong fluorescence when excited by 365 nm UV light, as shown in their photographs under UV (insets in Fig. 11a). Without organic solvent, the motion of TPE moieties was efficiently restricted and the AIE fluorescence was completely activated. Their quantum yield was measured as 12.7%. As far as we are aware, this is the first *in situ* study of the appearance of AIE fluorescence along with the polymersomes formation.

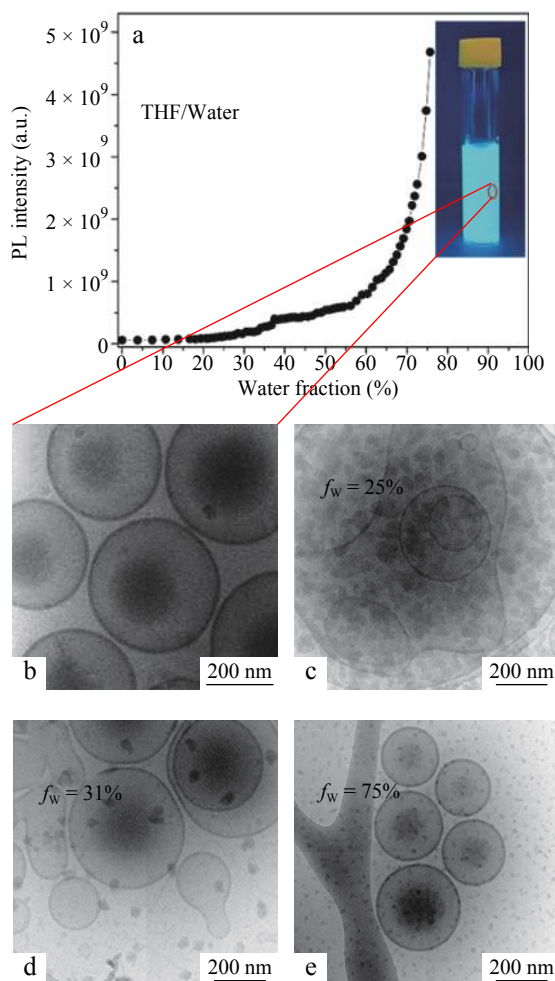


Fig. 11 (a) Evolution of fluorescence intensity as a function of water content during the polymersomes formation; (b) The polymersomes finally obtained in water imaged by cryo-EM; The morphologies of self-assemblies of PEG₄₅-*b*-P(TPE-TMC)₁₃ in THF/water mixture with water content of (c) $f_w = 25\%$, (d) $f_w = 31\%$, and (e) $f_w = 75\%$ (Reproduced with permission from Ref. [47]; Copyright (2018) American Chemical Society)

AIE Amphiphilic Polymers with AIEgens in the Main-chain

Click reaction is a popular and highly efficient reaction often used in chemistry and biochemistry. It was used to post-functionalize the block copolymer as discussed in section *AIE amphiphilic block copolymers prepared by post-functionalization*. It can also be used to directly synthesize

the main-chain polymers. Recently, main-chain AIE amphiphilic block copolymers have been prepared by click reaction from two bi-functional monomers: TPE-type diazide and alanine-containing diyne.^[48] These polymers, P(TPE-alanine), are also amphiphilic, where the amide groups on the side chain and triazole segments on the main chain act as hydrophilic part, and the TPE in the main chain and the ester groups in the side chain as hydrophobic part (Fig. 12A). Notably, unlike the conventional AIE amphiphilic block copolymer containing TPE side chains, which has almost no fluorescence in diluted solution, the P(TPE-alanine) exhibited weak fluorescence emission at 463 nm in solution (Fig. 12B). This is because the intramolecular rotation of TPE moieties would be already restricted to some extent as they were in the polymer backbone. Using nanoprecipitation method, P(TPE-alanine) self-assembled into polymersomes and other morphologies with strong aggregation-induced emission. In THF/H₂O mixture, at water fraction (f_w) of 50% (Fig. 12C-a2), polymersomes were first formed by P(TPE-alanine). When the water content increased to 60%, the polymersomes tended to fuse to form short nanofibers (Figs. 12C-b2 and 12D). At $f_w = 80\%$, the worm-like fibers started to form, and the fibers changed to long and thick ones at $f_w = 90\%$ (Figs. 12C-c2 and 12C-d2). The fluorescence microscope images showed that all the aggregates were highly emissive with greenish-blue color (Figs. 12C-a1 to 12C-d1). This work is another nice example of *in situ* visualization of aggregation-induced emission during the self-assembling process.

AIE VESICLES FORMED BY SUPRAMOLECULAR AND SUPRAMACROMOLECULAR AMPHIPHILES

Supra-amphiphiles are constructed by two or more molecular/macromolecular building blocks *via* noncovalent interactions, such as electrostatic interaction, hydrogen bonding, host-guest interaction, π - π interaction, *etc.*^[49–53] The strategy of constructing AIE supra-amphiphiles provides another interesting way toward AIE vesicles that does not need direct synthesis of amphiphilic molecules or polymers with different hydrophobic/hydrophilic ratios, which is sometimes tedious and complicated.^[54–63]

AIE Supramolecular Amphiphiles

Complexation by host-guest interaction

In 2016, Sessler *et al.* successfully constructed multilamellar AIE vesicles with supra-amphiphile **WC4P>1** (see Fig. 13).^[54] Under neutral or basic conditions, the complexation of TPE-containing pyridine bis-*N*-oxide (**1**, guest molecule) and a water-soluble calix[4]pyrrole with aryl periphery functionalized by four COO[−] groups (**WC4P**, host molecule) could form neutral supramolecular complex **WC4P>1**. The host-guest interaction with a combination of hydrophobic interactions and hydrogen bonds stabilized the complex with the guest binding deeply embedded into the aromatic cavity of calix[4]pyrrole receptor (Fig. 13a). The guest molecule **1** is an amphiphilic molecule with a critical aggregation concentration (CAC) of 1.2×10^{-4} mol·L^{−1}, which formed spherical micelles above CAC. The supra-

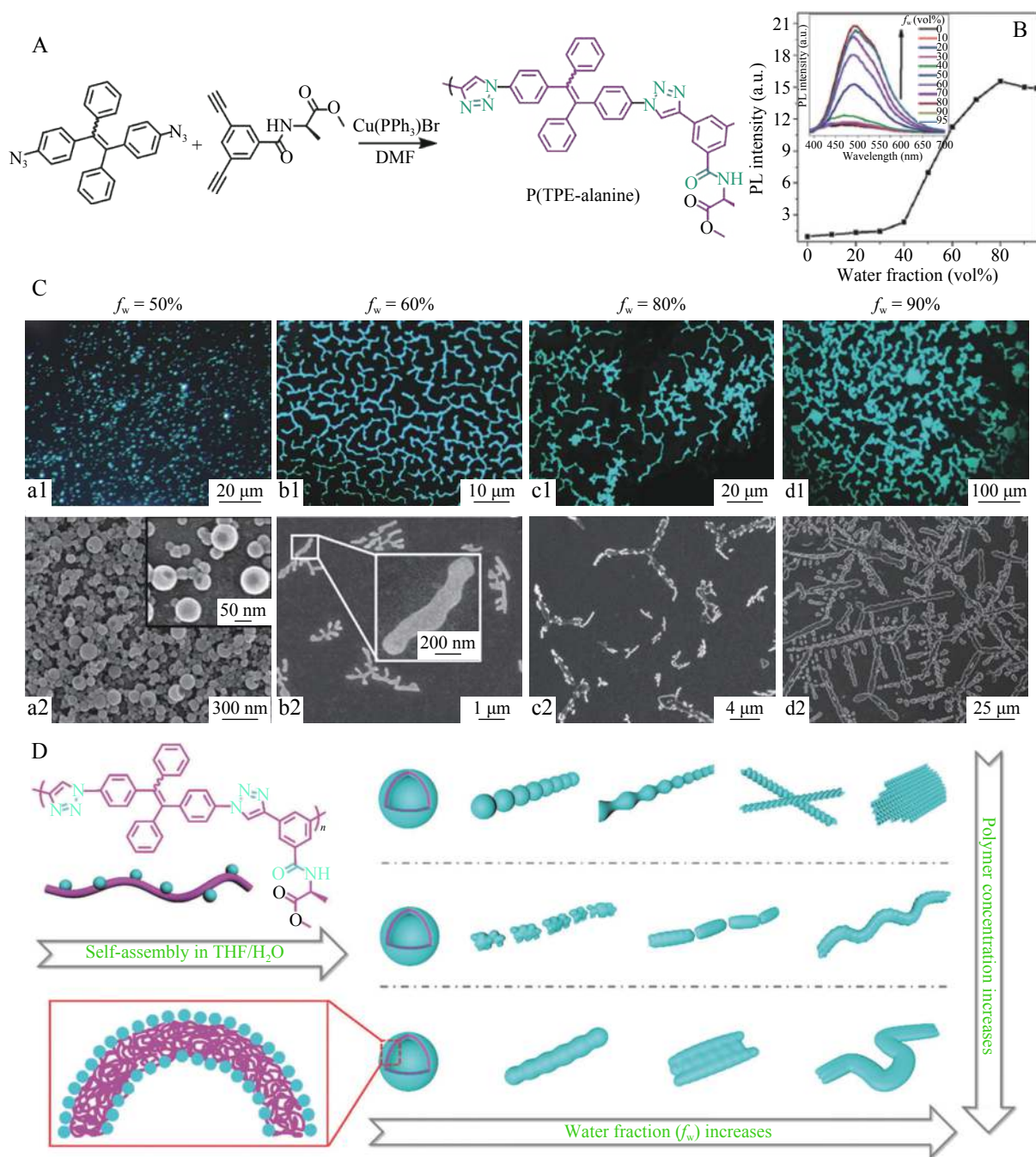


Fig. 12 (A) Synthetic route to P(TPE-alanine) by click polymerization (the purple-labeled part is hydrophobic, and blue-green ones are hydrophilic segments); (B) PL spectra of P(TPE-alanine) in pure THF and THF/H₂O mixtures. Excitation wavelength: 328 nm; (C) Fluorescence (a1–d1) and SEM (a2–d2) images of P(TPE-alanine) obtained from the THF/H₂O mixture at different volume ratios: (a1, a2) 5:5; (b1, b2) 4:6; (c1, c2) 2:8; (d1, d2) 1:9. The insets in a2 and b2 show the magnified SEM images. Solution concentration: $5 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$; (D) Schematic representation of the self-assembly and morphology transition processes of P(TPE-alanine) in the THF/H₂O mixture at different concentrations and f_w (Reproduced with permission from Ref. [48]; Copyright (2018) The Royal Society of Chemistry)

molecular complex **WC4P**⊃**1** is a bola-type supra-amphiphile suitable for vesicle formation and has a lower CAC ($5.3 \times 10^{-6} \text{ mol} \cdot \text{L}^{-1}$) than that of **1**. Multilamellar AIE vesicles with average diameter of 148 nm were formed when 2 equiv. of **WC4P** ($c = 3.0 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$) was added to the solution of guest molecule **1** ($c = 1.5 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$) as shown in Figs. 13(b)–13(d). Both fluorescence spectra and fluorescence microscopy image showed that the vesicles exhibited

strong blue fluorescence (Fig. 13e). They explained that the strong fluorescence was AIE type generated from the restricted rotation of the phenyl rings of TPE moieties when they are constrained in the multilayers of the vesicles.

More interestingly, the introduction of COO^- groups and host-guest interaction (non-covalent interaction) in the complex endow the vesicles with pH responsive property. As shown in Fig. 14(b), UV absorbance got decreased after

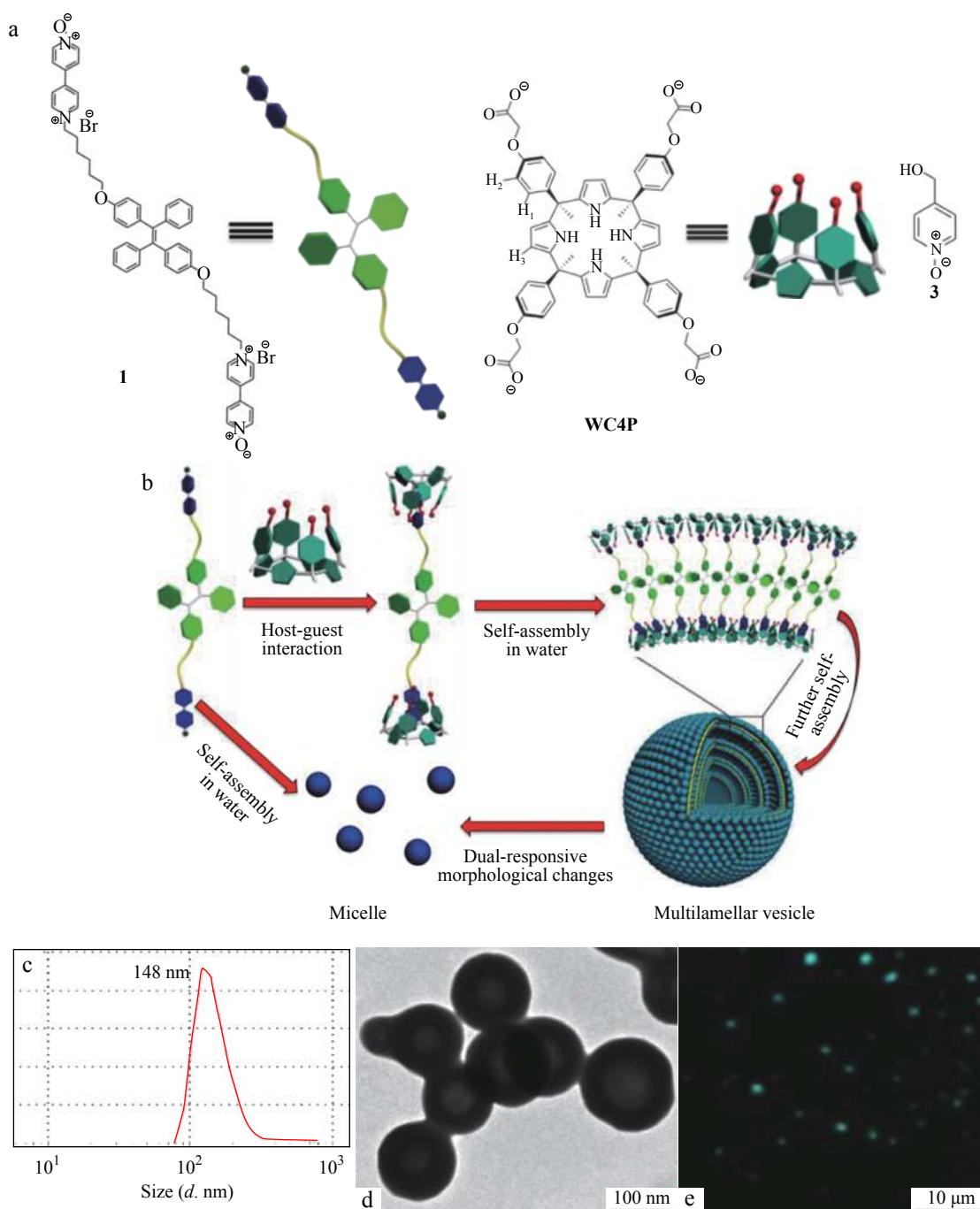


Fig. 13 (a) Molecular structures of host molecule **WC4P** and guest compounds **1** and **3**; (b) Illustration of self-assembly of **WC4P**>**1**, especially, a dual-responsive morphological changes from vesicles to micelles upon pH variation or the addition of competitive guest compound **3**; (c) DLS curve for self-assembled vesicles at 25 °C; (d) The TEM image of self-assembled vesicles; (e) Fluorescence microscope image of the self-assembled vesicles ($c_{\text{WC4P}} = 3.0 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$, $c_1 = 1.5 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$) (Reproduced with permission from Ref. [54] Copyright (2016) The Royal Society of Chemistry)

changing the pH from 7.4 to 4.0 or adding the competitive guest **3**. Authors used this system to monitor the release of a non-fluorescent molecule (gemcitabine) by testing the evolution of fluorescent intensity under acidic condition. In fact, the increase in absorbance intensity of gemcitabine (released) was accompanied by a decrease in fluorescence intensity of the vesicles (AIE deactivation) upon the addition of acid or of compound **3** (Fig. 14). This is especially useful for monitoring the release of non-fluorescence agents which

is need to be followed by optical means.

Complexation by electrostatic interaction

AIE supra-amphiphiles constructed *via* electrostatic interaction could also be employed to fabricate the AIE vesicles in aqueous media.^[54–59] In 2016, Yan and co-workers developed a TPE derivative carrying four water soluble carboxylate heads (TPE-BPA), as shown in Fig. 15(a).^[55–57] TPE-BPA was highly soluble and emitted weakly in water. The zeta potential of TPE-BPA water solution was –17 mV

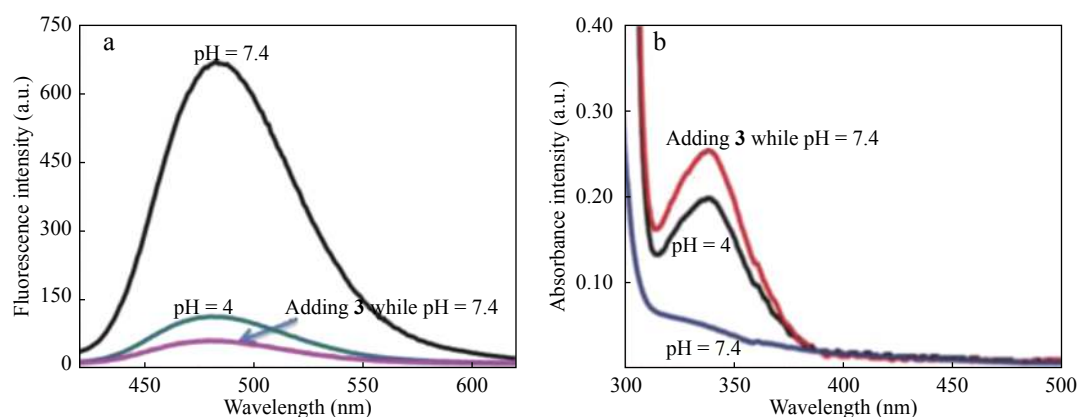


Fig. 14 (a) Fluorescence emission spectra of the supramolecular vesicle solution (**WC4PDI** with gemcitabine inside the vesicle), $\lambda_{\text{ex}} = 340 \text{ nm}$; (b) UV-Vis spectra of the same solution showing the absorbance intensity of gemcitabine upon the addition of acid. The concentration of the **WC4PDI** is $8.0 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$. (Reproduced with permission from Ref. [54]; Copyright (2016) The Royal Society of Chemistry)

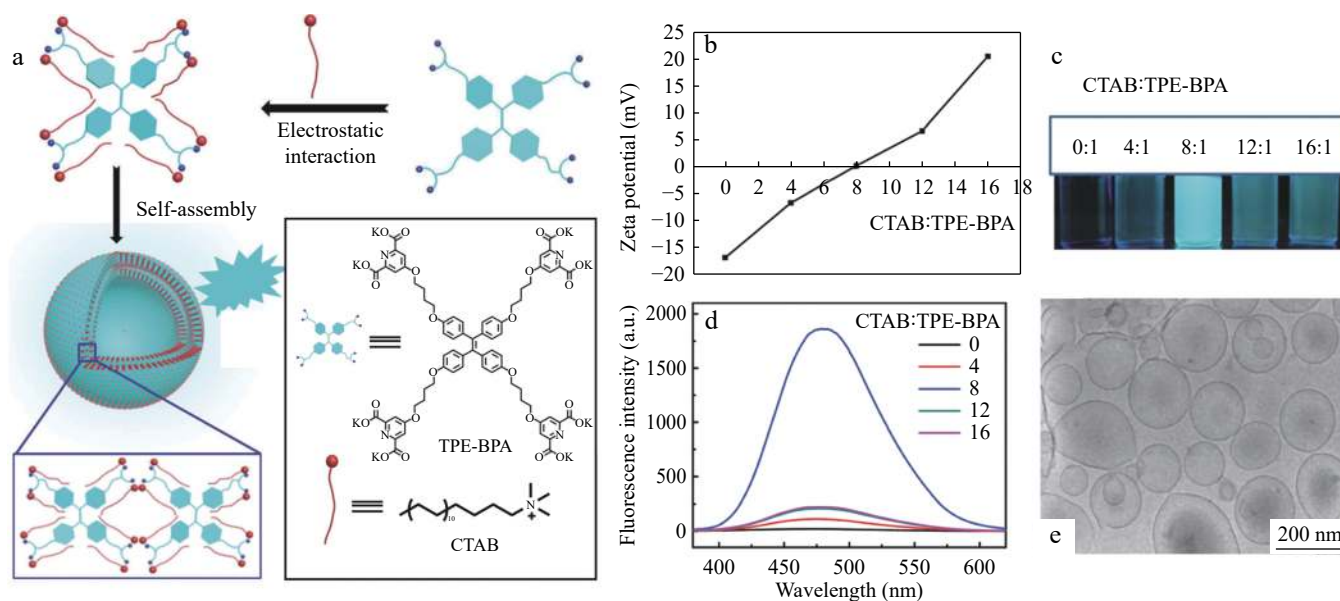


Fig. 15 (a) Formation of vesicles with TPE-BPA and cationic hydrocarbon chain, as well as the molecular structures of TPE-BPA and CTAB; (b) Variation of the zeta potential with different CTAB:TPE-BPA molar ratios; (c) Photo of the CTAB:TPE-BPA solution under a 365 nm UV lamp at various molar ratios; (d) Fluorescence spectra of the CTAB:TPE-BPA samples with different molar ratios; (e) Cryo-TEM image of the vesicles formed by TPE-BPA@8CTAB. (Reproduced with permission from Ref. [55]; Copyright (2016) American Chemical Society)

(Fig. 15b). Upon addition of cationic hydrocarbon chain cetyltrimethylammonium bromide (CTAB), a zero potential could be obtained at the CTAB:TPE-BPA molar ratio of 8:1, leading to a neutral supramolecular complex TPE-BPA@8CTAB via the electrostatic interaction (Fig. 15b). AIE vesicles were formed by the neutral supramolecular complex TPE-BPA@8CTAB (Fig. 15e), whose hydrophobic ratio was significantly higher than that of TPE-BPA because of CTAB. As shown in Fig. 15(c), the fluorescent intensity greatly increased after the addition of CTAB and showed the maximum emission at the molar ratio of CTAB:TPE-BPA equal to 8:1.

Then they used the TPE-BPA@8CTAB vesicle to fabricate folate acid (FA) modified AIE vesicles FA-HSFVs by adding tetraethoxysilane (TEOS) and FA modified (3-amino-

propyl)triethoxysilane (FA-APTS) into the aqueous solution of vesicles (Fig. 16).^[56] The introduction of FA into vesicle surface endowed the vesicles with targeting ability toward HeLa cells (Fig. 16A). In addition, TEOS and FA-APTS added additional thickness to the membrane, which could increase the stability of vesicles. Bright blue fluorescence from HeLa cells was observed after 24 h incubation with FA-HSFVs as the FA receptors highly expressed on the surface of tumor cell (Figs. 16B-a and 16B-c). By contrast, the fluorescence was extremely weak when the vesicles were incubated with human normal hepatic cell line LO2 cells (Figs. 16B-b and 16B-d). MTT colorimetric bioassay results indicated the negligible cell toxicity of FA-HSFVs for HeLa cells (Fig. 16C). However, the DOX-loaded vesicles FA-HSFVs presented effective ability in killing HeLa cells (Fig. 16D).

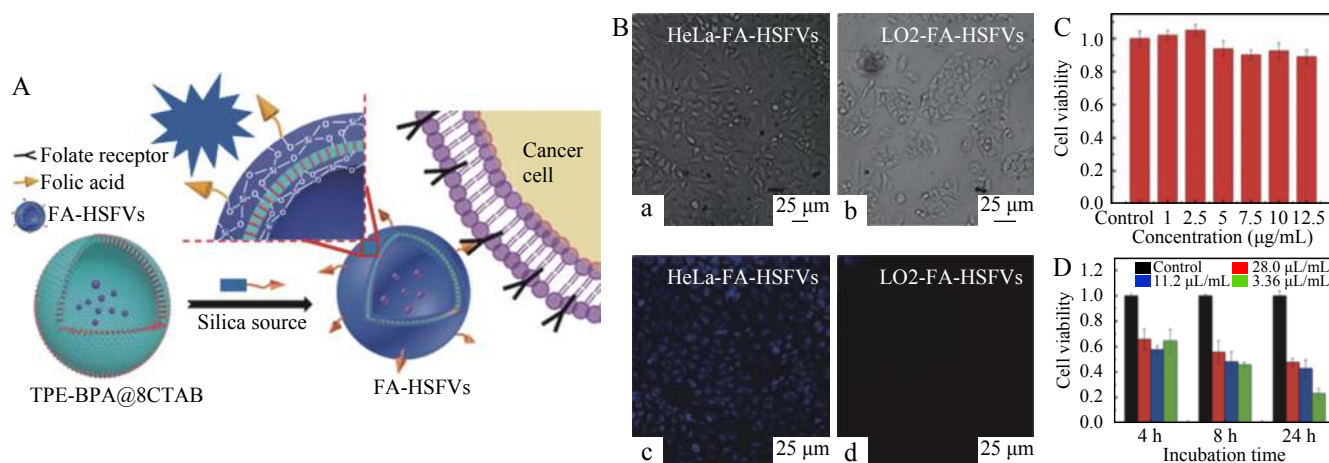


Fig. 16 (A) Preparation of folic acid modified silicated fluorescent supramolecular vesicles (FA-HSFVs) with TPE-BPA@8CTAB vesicles, and their targeted combination with cancer cells; (B) CLSM images of cells with vesicles: bright field images of (a) HeLa and (b) LO2 cells with FA-HSFVs, and fluorescence images of (c) HeLa and (d) LO2 cells with FA-HSFVs; (C) Cytotoxicity of FA-HSFVs with different concentrations in HeLa cells; (D) Cell viability of HeLa cells incubated with DOX-loaded FA-HSFVs over time. The cell viability decreases with lowering the silica source concentration. The legends represent the amount of tetraethoxysilane (TEOS) added during the preparation of DOX loaded FA-HSFVs. [TEOS] = [FA-APTS] = 28.0, 11.2, and 3.36 $\mu\text{L}\cdot\text{mL}^{-1}$, $T = 37^\circ\text{C}$. (Reproduced with permission from Ref. [56]; Copyright (2017) American Chemical Society)

With the same strategy, they successfully created another example of supramolecular complex TPE-BPA@8MChCl by changing the cationic hydrocarbon chain from CTAB to myristoylcholine chloride (MChCl) (Fig. 17a).^[57] TPE-BPA@8MChCl supra-amphiphile could self-assemble into fluorescent vesicles with diameter of 110 nm (Fig. 17b).

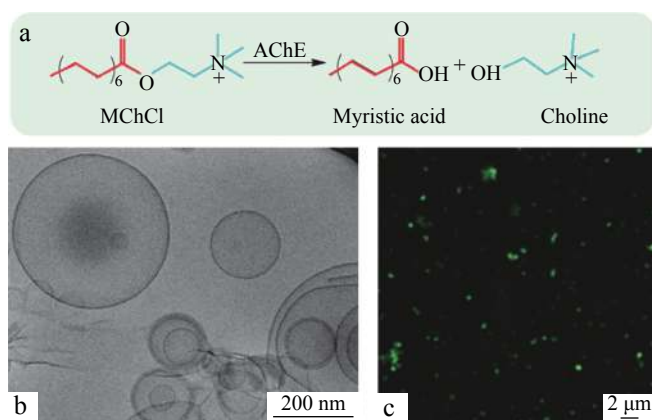


Fig. 17 (a) Molecular structure of MChCl and its hydrolyzed route by acetylcholinesterase; (b) Cryo-TEM image of the vesicles formed by TPE-BPA@8MChCl; (c) CLSM image of the vesicles formed in TPE-BPA@8MChCl system (Reproduced with permission from Ref. [57]; Copyright (2016) American Chemical Society)

Because MChCl moiety can be hydrolyzed by acetylcholinesterase (AChE) which is overexpressed in the Alzheimer's disease (Fig. 17a), TPE-BPA@8MChCl vesicles possess enzyme responsive properties. The vesicles disassembled in the presence of cholinesterase and made their fluorescence intensity decreased. Real-time confocal laser scanning microscopy (CLSM) images showed that the regular spheres (small vesicles) with high fluorescence intensity in the absence of cholinesterase (Fig. 18A-a) changed to

irregular ones when treated with cholinesterase in 30 min (Fig. 18A-b), and to bar-like aggregates until completely vanished with the increase of the treatment time (Fig. 18A-d). Furthermore, they loaded tacrine, a typical water-soluble acetylcholinesterase inhibitor for the clinical treatment of Alzheimer's disease, into the fluorescent vesicles. The fluorescence intensity of tacrine-loaded vesicles did not show any change after 2 h in the absence of enzyme (Fig. 18B, red line), while the fluorescence intensity of the solution with unloaded vesicles showed a great decrease in the presence of enzyme (Fig. 18B, black line). However, the fluorescence intensity of tacrine-loaded vesicles reduced by only about 20% after the addition of cholinesterase, which is much lower than that of unloaded system (Fig. 18B, blue line versus black line). This suggested that a part of vesicles were disrupted with tacrine's releasing, and meanwhile, the released tacrine inhibited the activity of cholinesterase to prevent further disassembly of the tacrine-loaded vesicles (Fig. 18C). Consequently, some vesicles maintained their morphologies due to the inactivation of cholinesterase by released tacrine.

Yan's group also reported two-photon fluorescent vesicles based on supramolecular complex $\text{Zn}(\text{TPA-1})_2$ (Fig. 19a).^[58] The AIE molecule potassium triphenylamine carboxylate (PTA-1) and bivalent Zn^{2+} ions interacted together *via* electrostatic interaction to generate the supramolecular complex $\text{Zn}(\text{TPA-1})_2$, which further self-assembled into vesicles (Fig. 19b). Since TPA-1 molecules were firmly confined in the vesicle membrane, the obtained vesicles displayed strong green fluorescence (Fig. 19c). Moreover, the two-photon fluorescent vesicles could be used for cell imaging because of the high penetration of exciting light (Fig. 20). HaLa cells were incubated with the aqueous dispersion containing $\text{Zn}(\text{TPA-1})_2$ vesicles for 12 h. The vesicles could cross the negatively charged membrane to enter HaLa cells. Green and red emissions were obtained in the channel of 420–475 and 500–550 nm, respectively, by excit-

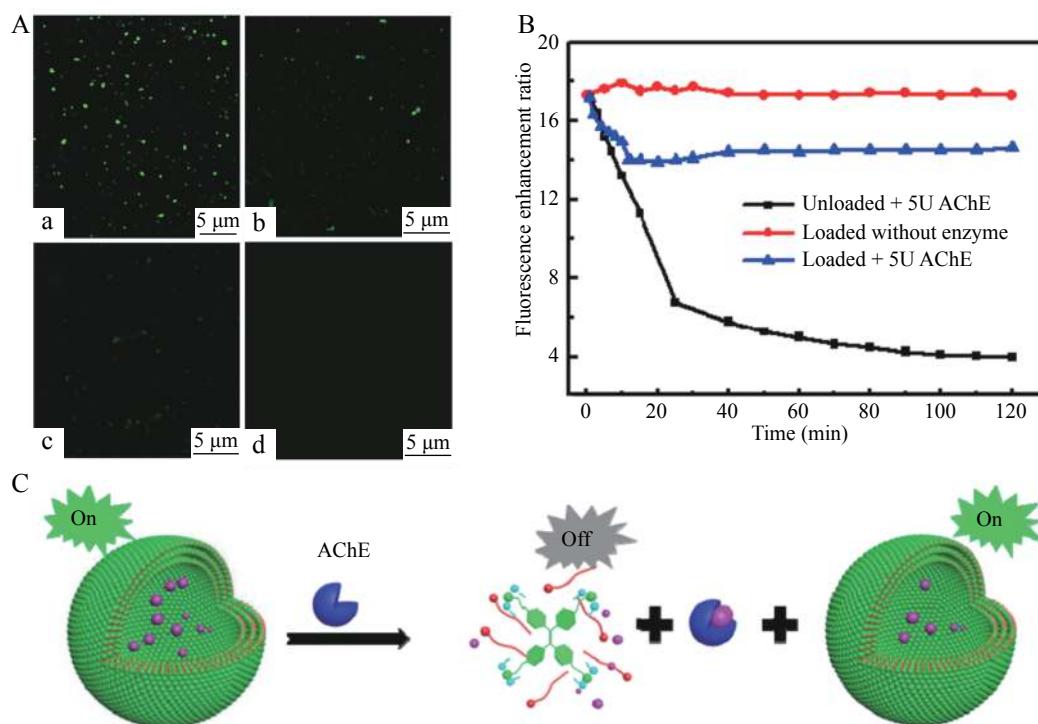


Fig. 18 (A) Real-time CLSM images of the TPE-BPA@8MChCl system in the presence of 1 U·mL⁻¹ AChE for (a) 0, (b) 1.5, (c) 3, and (d) 24 h; (B) Fluorescence variation of vesicle solution in the presence or absence of 5 U·mL⁻¹ AChE over time; (C) Illustration of the partial disassembly of the fluorescent vesicles loaded with tacrine (pink spheres) in presence of the enzyme AChE (Reproduced with permission from Ref. [57]; Copyright (2016) American Chemical Society)

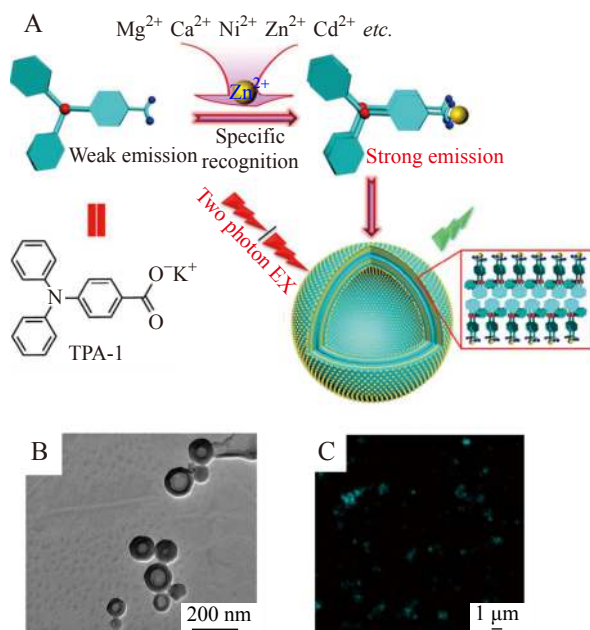


Fig. 19 (A) Chemical structures of TPA-1 and illustration of the self-assembly of TPA-1/Zn²⁺ fluorescent vesicle; (B) TEM image of the vesicles formed in the PA-1/Zn²⁺ systems; (C) CLSM image for the vesicles formed in the TPA-1/Zn²⁺ system excited under 405 nm laser (Reproduced with permission from Ref. [58]; Copyright (2018) American Chemical Society)

ing the vesicles with a mode-locked titanium-sapphire two-photons laser source set at wavelength 800 nm (Figs. 20a and 20b). Besides, the Zn(TPA-1)₂ vesicle showed no obvious toxicity to HeLa cells at the concentration from

0 μg·mL⁻¹ to 12.5 μg·mL⁻¹. Thus, Zn(TPA-1)₂ AIE vesicles are expected to be promising two-photon fluorescent probes for cell imaging. Moreover, the cancer cell viability significantly decreased when treated with the Zn(TPA-1)₂ vesicles encapsulating water-soluble antimetabolite compound 5-fluorouracil (5-Fu).

Recently, Jiang and Li *et al.* reported another cation-anion interaction-directed formation of functional AIE vesicles based on TPE derivative with multiple imidazolium moieties and alkyl carboxylate anions (TPEI-C8).^[59] This system exhibited highly specific nucleus imaging capacity in living cells because of the aggregation-induced strong fluorescence emission of TPEI-C8 in cell nucleus.

AIE Supramacromolecular Amphiphiles

In 2014, Bu *et al.* reported an example of AIE supramacromolecular amphiphiles through the host-guest chemistry.^[60] Firstly, they synthesized a crown ether functionalized main chain poly(tetraphenylethene) (AP-TPE, host polymer) via the Sonogashira cross-coupling reaction of dibenzo-24-crown-8-(DB24C8)-substituted 1,4-diiodobenzene and 1,2-bis(4-ethynylphenyl)-1,2-diphenylethene as shown in Fig. 21(A). Interestingly, the complexation of the host polymer, AP-TPE, and the guest molecule, C12-2H⁺·PF₆⁻, with dibenzylammonium salts units (DBAs) at two extremities of hydrocarbon C12 did not form vesicular nanostructure in solution. They found that spherical polymersomes could be created only when the solution of AP-TPE and C12-2 (neutral guest molecules) was successively treated with acid and base. Specifically, after treating the initial solution of AP-TPE and C12-2 with 2.0 equiv. of HCl (referred to the

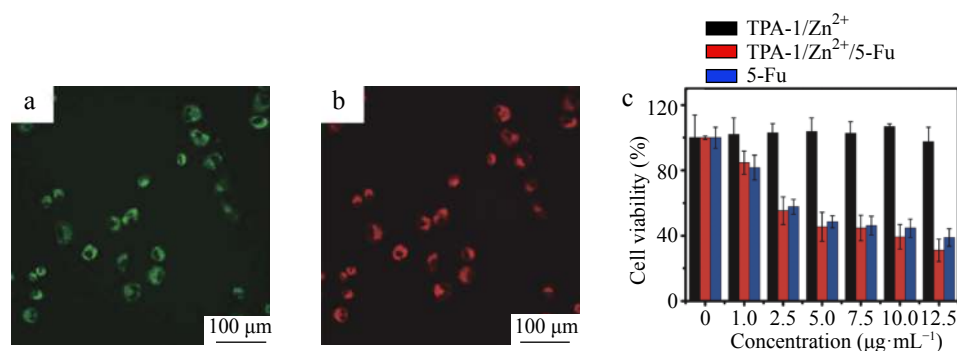


Fig. 20 Two-photon Hela cell image following a 12 h treatment with TPA-1/Zn²⁺ (50 μmol·L⁻¹, 2:1): (a) emission wavelength from 420 nm to 475 nm and (b) Emission wavelength from 500 nm to 550 nm; (c) Viabilities of Hela cells in the presence of TPA-1/Zn²⁺ (black), TPA-1/Zn²⁺/5-Fu (Red), and 5-Fu (Blue), as assayed by MTT (Reproduced with permission from Ref. [58]; Copyright (2018) American Chemical Society)

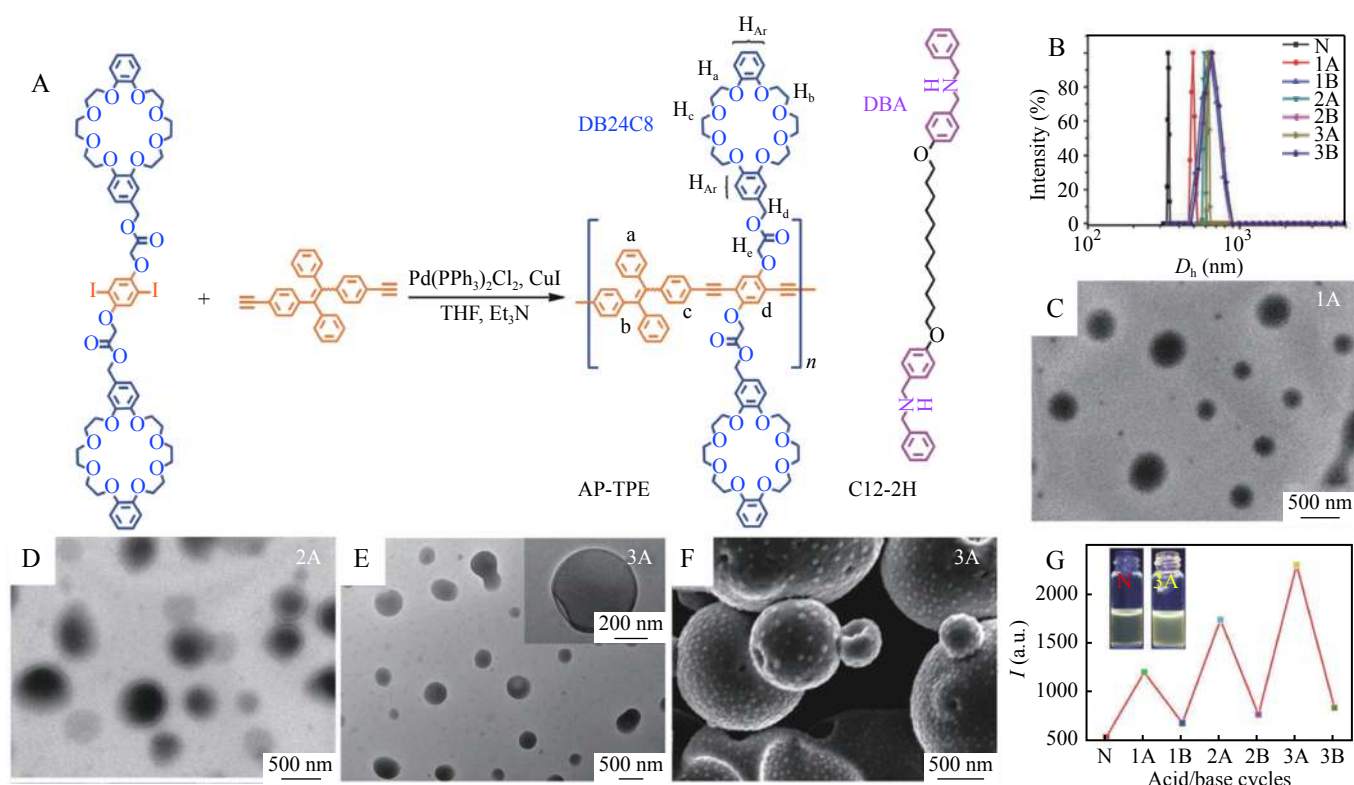


Fig. 21 (A) The chemical structure of AP-TPE and C12-2H; (B) DLS plot of AP-TPE and C12-2 in THF treated with HCl and NaOH repeatedly; (C–E) TEM images of AP-TPE and C12-2 after the first, second, and third acidification; (F) SEM images of AP-TPE and C12-2 after the third acidification; (G) The fluorescence intensity of AP-TPE/C12-2 in THF treated with HCl and NaOH, repeatedly. N, A, and B denote the neutral solution and its acid and base treatments, respectively. 1, 2, and 3 denote the sequence of the acid-base cycle (Reproduced with permission from Ref. [60]; Copyright (2015) The Royal Society of Chemistry)

monomer concentration), the diameter (D_h) of aggregates with solid cores increased from 338 nm (Figs. 21B and 21C) to 492 nm (Figs. 21B and 21D) due to the interaction between DB24C8 moieties in polymer and DBA groups from the guest molecules C12-2. Moreover, the diameter of aggregates did not return to the original value after treated with NaOH, but grew to 631 nm. After the second acid-base cycle, the solid aggregates transformed to polymersomes with diameters ranging from 400 nm to 600 nm (Figs. 21E and 21F). The diameter would further increase (from 500 nm to 700 nm) after the third acid-base cycle. It was proposed that, after the second acid-base cycle, the interaction

between DB24C8 and DBA along with the salting-out effect (acid-base reaction generated NaCl) modified the solvophilic-solvophobic effect which would be responsible for morphological transition from polymer solid spheres to polymersomes. The main chain polymer AP-TPE in pure THF also exhibited weak fluorescence (Fig. 21G).^[48] The fluorescence was enhanced after self-assembling (Fig. 21G), and a stepwise enhancement of the fluorescence was found when the system was treated with acid-base cycle (Fig. 21G).

In 2015, Huang *et al.* reported highly emissive AIE vesicles based on supramacromolecular amphiphiles which is composed of a side chain TPE-containing guest polymer 1

and a PEG-containing host polymer **2** as shown in Fig. 22.^[61] The random side chain copolymer **1** was prepared from styrene, TPE-methacrylate, and 4-vinylbenzyl chloride *via* free radical polymerization followed by a post-coupling reaction with 1-methyl-[4,4'-bipyridin]-1-ium iodide to add the guest moieties bipyridinium (Fig. 22a). Meanwhile, the host polymer **2** with the water-soluble PEG and a bis(*m*-phenylene)-32-crown-10 (BMP32C10) host moiety bearing two $-\text{COO}^- \cdot \text{NH}_4^+$ groups was synthesized (Fig. 22b). Supramacromolecular amphiphiles were afforded by mixing polymers **1** and **2** due to the recognition of BMP32C10 in host polymer **2** and the bipyridinium segments in guest polymer **1** (Fig. 22b). When water was added into the solution of **1/2** complex in DMF, different morphologies of self-assemblies were obtained depending on the relative concentration of **1/2**. When the concentrations of **1** and **2** in aqueous solution were fixed at $1.71 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ ($5.30 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ for bipyridinium units) and $1.71 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ ($1.71 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ for crown ether moieties), respectively, vesicles with average diameter of 700 nm were formed (Fig. 22c). With the increasing proportion of the host polymer **2**, the morphology of self-assemblies transformed from vesicles to worms (Fig. 22d), then to micelles (Fig. 22e).

Moreover, the obtained vesicles showed pH responsive property. At lower pH, the $-\text{COO}^- \cdot \text{NH}_4^+$ groups changed

into carboxylic acid form, weakening the complexation between the host and guest polymer. Consequently, the vesicles were destroyed. The release experiment of hydrophilic drug gemcitabine clearly showed the UV absorption intensity of gemcitabine (no fluorescence) released in water increased and the fluorescence intensity of the AIE supramacromolecular vesicles decreased steadily upon the pH lowering (Figs. 23a and 23b). Similar to the AIE supramolecular vesicles reported by Sessler *et al.*,^[54] the release of the encapsulated drugs from the AIE supramacromolecular vesicles could also be monitored by the fluorescence intensity decrease due to the deactivated AIE effect with vesicle destruction.

In 2016, AIE supramacromolecular vesicles based on inclusion complexation of β -cyclodextrin and tyloxapol were reported by Yuan *et al.* (Fig. 24a).^[62] Tyloxapol is an amphiphilic non-ionic liquid polymer of the alkyl aryl polyether alcohol type (4-(1,1,3,3-tetramethylbutyl)phenol polymer with formaldehyde and oxirane). Initially, the water solution of tyloxapol was transparent (Fig. 24b), whereas turbid solution with a Tyndall effect was produced upon the addition of β -CD, suggesting formation of nanostructure in system (Fig. 24c). When the ratio of tyloxapol and β -CD was 1:7 or 1:14, the supramacromolecular complexes self-assembled into vesicles with average diameter of 500 nm (Fig. 24e). The

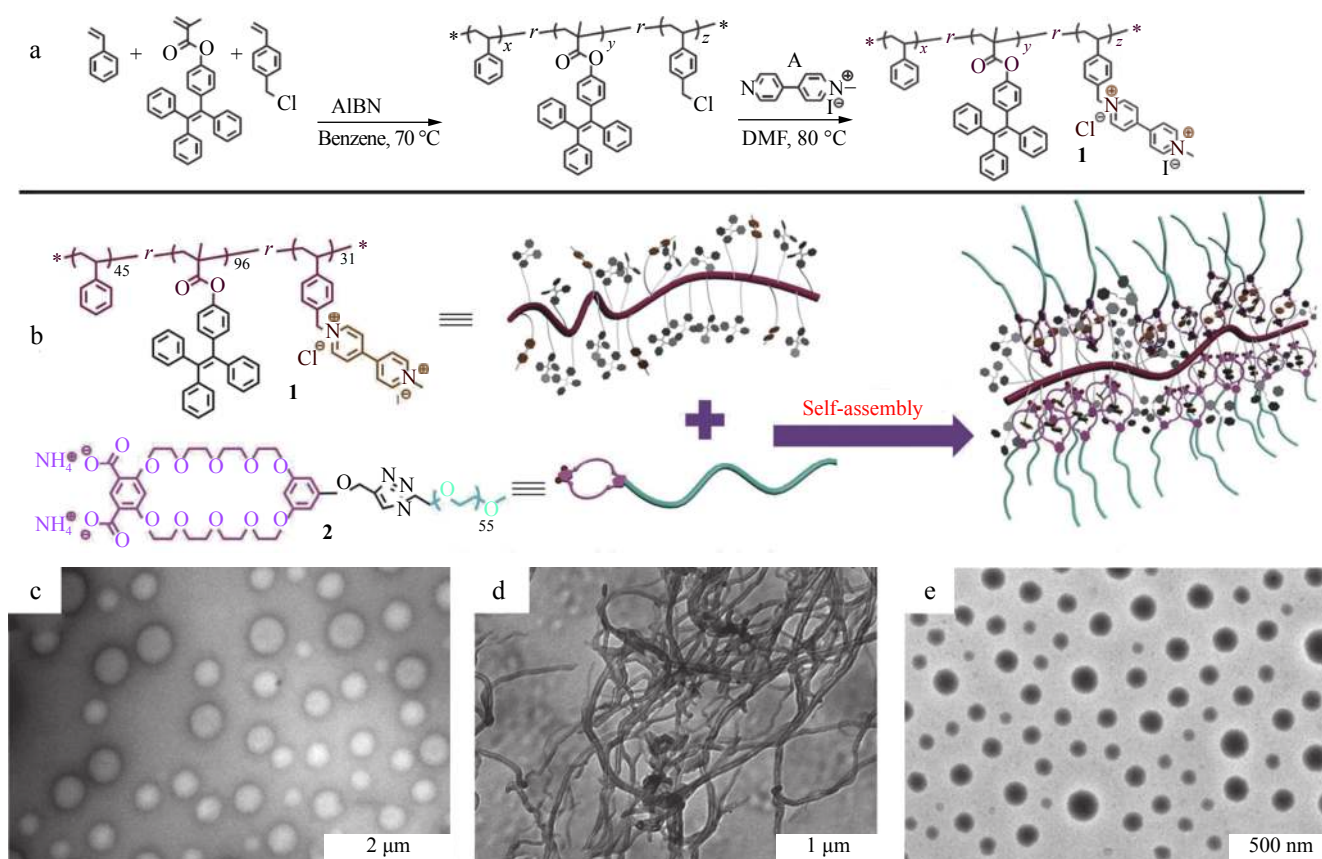


Fig. 22 (a) Synthetic route of polymer **1**; (b) Chemical structures of **2** and illustration of the supramolecular amphiphilic graft copolymer formed from self-assembly of **1** and **2**; (c) TEM image of vesicles formed with $[\mathbf{1}]/[\mathbf{2}] = 1.71 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}/1.71 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ in water; (d) TEM image of worm-like micelles formed with $[\mathbf{1}]/[\mathbf{2}] = 1.71 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}/3.41 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ in water; (e) TEM image of spherical micelles formed with $[\mathbf{1}]/[\mathbf{2}] = 1.71 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}/5.12 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ in water (Reproduced with permission from Ref. [61]; Copyright (2015) The Royal Society of Chemistry)

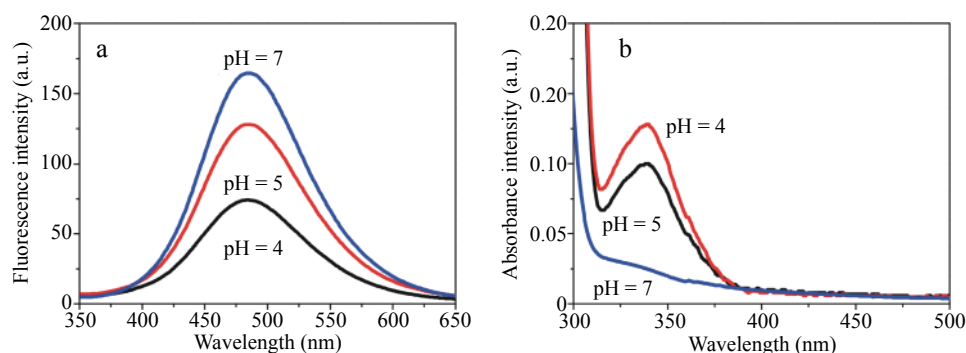


Fig. 23 (a) Fluorescence emission spectra and (b) UV-Vis spectra of the dispersion of AIE supra-macromolecular vesicles shown in Fig. 22 (with gemcitabine encapsulated inside) as a function of pH (Reproduced with permission from Ref. [61]; Copyright (2015) The Royal Society of Chemistry)

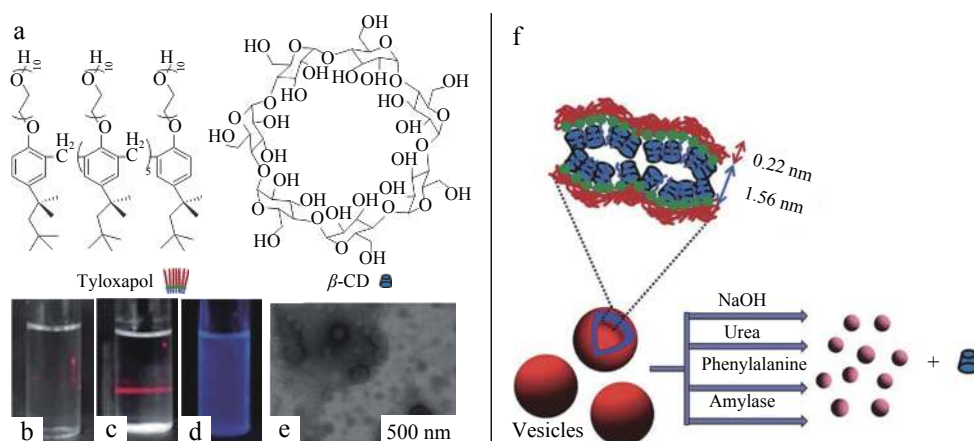


Fig. 24 (a) The chemical structure of tyloxapol and β -CD; (b) Photo of selected samples of 2 mmol·L⁻¹ tyloxapol; (c) Strong Tyndall effect of 2 mmol·L⁻¹ tyloxapol/14 mmol·L⁻¹ β -CD vesicle solutions (tyloxapol: β -CD = 1:7); (d) The vesicle solution under 365 nm UV irradiation; (e) TEM images of formed vesicle (tyloxapol/ β -CD = 1:14); (f) Schematic diagram of the possible models of tyloxapol and β -CD/tyloxapol aggregates and their responsiveness to various environmental conditions (Reproduced with permission from Ref. [62]; Copyright (2015) The Royal Society of Chemistry)

compositions of the complexes did not contain the normal AIEgens, but a strong fluorescence was observed for the vesicle dispersion of β -CD/tyloxapol (with molar ratio of 1:14) under 365 nm UV-irradiation as shown in Fig. 24(d). They suggested that the alkyl chain of tyloxapol interacted with the hydrophobic cavity of β -CD to form inclusion complexation, which made the benzene rings packed more closely. Consequently, a stronger π - π interaction between benzene rings resulted in the AIE phenomenon. Herein, the linear oligomer of phenolic resin (with the phenol rings joined by methylene radicals) played the role of AIEgen. Moreover, it seemed that environmental alkaline stimuli like urea, phenylalanine, amylase, and NaOH could destroy the vesicles with the decrease of fluorescence intensity due to their strongly competitive recognition and electrostatic repulsion (Fig. 24f).

Atomically precise metal nanoclusters (NCs) represent a new kind of luminescent materials. However, NCs usually display luminescence quenching in a single-molecule state in solution. Inspired by the AIE phenomenon of organic AIEgens, the AIE methodology has also been used to lighten the luminescence of metal NC materials. Sun *et al.* fabricated AIE vesicles based on silver Ag(I) NCs (Ag₆-NCs) and pos-

itively charged polyethyleneimine (PEI).^[63] For example, PEI with $M_w = 7.5 \times 10^5$ Da (PEI-1) and $M_w = 2.5 \times 10^5$ Da (PEI-2) could be complexed with Ag₆-NCs [(NH₄)₆[Ag₆(mna)₆], H₂mna = 2-mercaptosuccinic acid] *via* multiple electrostatic interaction to form AIE supramacromolecules/NCs hybrid vesicles (Fig. 25). They speculated that compact ordered self-assemblies restricted intramolecular vibration of the capping ligands on Ag₆-NCs to diminish the nonradiative decay, which ultimately induced an AIE phenomenon. Furthermore, the luminescent Ag₆-NCs/PEI nanovesicles were pH-responsive owing to the $-\text{COO}^-$ in Ag₆-NCs and showed a superior fluorescent sensing behaviour for the detection of Al³⁺ with a limit of detection low to 3 $\mu\text{mol}\cdot\text{L}^{-1}$ when compared with other ions, such as Na⁺, Ba²⁺, Zn²⁺, Pb²⁺, Ni²⁺, Mg²⁺, Co²⁺, Cu²⁺, and Fe³⁺ (Fig. 25). The coordination of Al³⁺ with N in PEI-1 provoked the disassembling of Ag₆-NCs/PEI-1 nanovesicles and consequently caused the deactivation of AIE fluorescence.

SUMMARY AND OUTLOOK

In this review, we summarized the recent development of fluorescent AIE vesicles according to the chemical structures

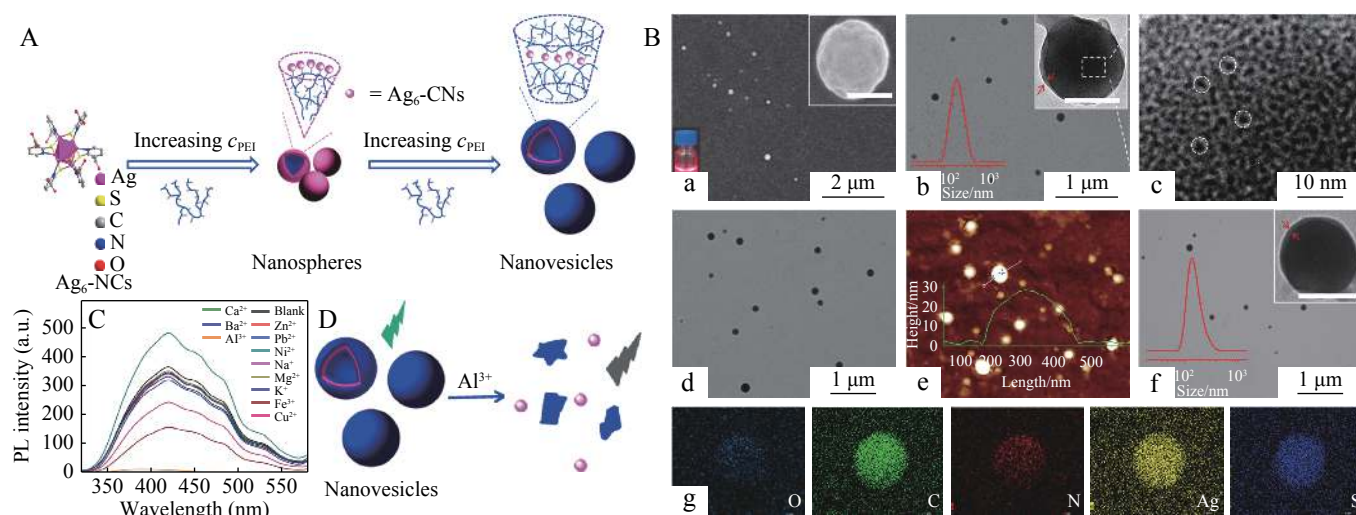


Fig. 25 (A) Schematic model of the formation of nanospheres and nanovesicles for Ag₆-NCs/PEI system with the increasing concentration $c_{\text{PEI-1}}$ or $c_{\text{PEI-2}}$; (B) (a) SEM, (b) TEM, (c) HR-TEM, and (e) AFM images of $c_{\text{Ag}_6\text{-NCs}}/c_{\text{PEI-1}} = 50 \mu\text{mol}\cdot\text{L}^{-1}/0.055 \mu\text{mol}\cdot\text{L}^{-1}$ vesicle; TEM images of (d) $c_{\text{Ag}_6\text{-NCs}}/c_{\text{PEI-1}} = 50 \mu\text{mol}\cdot\text{L}^{-1}/0.06 \mu\text{mol}\cdot\text{L}^{-1}$ and (f) $c_{\text{Ag}_6\text{-NCs}}/c_{\text{PEI-2}} = 50 \mu\text{mol}\cdot\text{L}^{-1}/1.5 \mu\text{mol}\cdot\text{L}^{-1}$; (g) SEM elemental mapping analyses of nanovesicles. The scale bar for all the inset magnified images is 100 nm. (C) PL spectra of nanovesicles with the addition of metal ions (Zn^{2+} , Pb^{2+} , Ni^{2+} , Na^+ , Mg^{2+} , K^+ , Fe^{3+} , Cu^{2+} , Ca^{2+} , Al^{3+} , and Ba^{2+} , $c_{\text{metal}} = 50 \mu\text{mol}\cdot\text{L}^{-1}$); (D) Schematic representation of the vesicle AIE vesicle-based probes for Al^{3+} ions (Reproduced with permission from Ref. [63]; Copyright (2018) American Chemical Society)

and architectures of the amphiphilic molecules that constitute the AIE vesicles, *i.e.*, small-molecule amphiphiles, amphiphilic polymers, and amphiphilic supramolecules/supramacromolecules. We can also classify the AIE vesicles simply into two categories, small-molecule AIE vesicles and AIE polymersomes, according to the molecular weights of the amphiphiles, *i.e.*, amphiphilic small molecules or amphiphilic polymers. Furthermore, both kinds of amphiphiles contain hydrophilic part and hydrophobic part with vesicle-forming ratios, which are connected either with covalent bond or *via* noncovalent interactions, such as host-guest interaction, electrostatic interaction, hydrogen bonding, *etc.* The emblematic tetraphenylethylene (TPE) was the most used AIEgen in all these AIE vesicles, which is the major component of the hydrophobic part in the amphiphilic small molecules or is incorporated in the side-chain or main-chain structure of the hydrophobic block of amphiphilic polymers. Other AIEgens like carbazole, triphenylamine carboxylate, linear oligomer of phenolic resin, and silver nanoclusters (Ag₆-NCs) have also been used to construct the AIE vesicles. The key issue is that amphiphilic molecules or polymers should provide the appropriate hydrophilic/hydrophobic balance.^[9] As amphiphilic polymers have hydrophilic block(s) and hydrophobic block(s) of high molecular weight, AIE polymersomes possess thicker membranes compared to those of small-molecule AIE vesicles and exhibit better stability. We have also shown that the stability of small-molecule AIE vesicles can be achieved by crosslinking the membranes.

Through these examples of AIE vesicles, we have shown that the membrane of vesicles offers an excellent nanoscale environment, with typical thickness of 5–30 nm, for the restriction of intramolecular motion of AIEgens. The fluorescence was activated or enhanced as the amphiphilic molecules self-assembled into the membrane of vesicles.

Thanks to AIE characteristics, AIE vesicles are considered as promising carriers not only for encapsulation and delivery of active substances, but also the innovative approaches for *in vivo* study of drug release and drug distribution. We reviewed AIE vesicles here as pH-responsive drug release systems,^[36,40–42,46,51,58,60] fluorescence-guided and targeted drug release systems,^[51,53,58] two-photo cell imaging agents,^[55] and fluorescent probes of different colors by FRET effect.^[36,38] For example, pH-responsive AIE vesicles have been developed with the incorporation of $-\text{COO}^-$ groups (protonation/deprotonation) or Schiff base groups (unstable bonds toward acidic conditions). Notably, the detection of drug release from AIE vesicles could be achieved by tracing the fluorescence intensity change of the fluorescent vesicles, without requiring the drugs to be fluorescent.^[51,58] In addition, AIE vesicles encapsulating another dye molecule (RhB or NR) have been used as an ideal platform for FRET systems because the membrane of AIE vesicles provide ideally confined space as well as the AIE property.^[36,38,64,65]

Nevertheless, the research on AIE vesicles is still at its beginning with limited examples published in the literature. Therefore, there are a lot of opportunities in this domain. The key issues for the design of efficient AIE vesicles for bioimaging and drug release systems reside in the targeting properties, the stimuli-responsive properties, and visible/NIR excitation and emission. Various functional moieties can be introduced into the vesicles to realize the targeted binding of tissue/cell and specific labelling and drug release. Different stimuli including chemical and physical stimuli can be developed in AIE vesicles. For example, physical stimuli, such as light or magnetic field, which show obvious advantage with the possibility of remote and precise control, are seldom studied for AIE vesicles. Furthermore, AIE vesicles which are excited and emit in visible/NIR range with narrow band emission and high fluorescence quantum

efficiency are highly desirable since they can avoid the harmful effects on the tissue/cells and can operate with deep penetration in biological tissue. We believe that further reinforced research on AIE vesicles will allow setting up an efficient AIE vesicle-based platform for bioimaging, diagnostics, and theranostics.

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