Chinese Journal of
POLYMER SCIENCE

REVIEW

https://doi.org/10.1007/s10118-018-2170-3
Chinese J. Polym. Sci. 2018, 36, 1103–1113

An "*In Vivo* Self-assembly" Strategy for Constructing Superstructures for Biomedical Applications

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Abstract The interfacing study of biopolymer and supramolecular chemistry enables a better understanding of fundamental biochemical processes and the creating of new high-performance biomaterials. In this review, we introduced an "*in vivo* self-assembly" strategy which means *in situ* construction of functional self-assembled superstructures in specific physiological or pathological conditions in cell, tissue or animal levels that exhibit diverse biomedical effects. By using this strategy, unexpected phenomena and insights, *e.g*, assembly/aggregation induced retention (AIR) effect have been demonstrated where the self-assembled nanostructures showed extraordinary enhanced accumulation and retention of therapeutics in targeted sites.

Keywords *In vivo* self-assembly; Supramolecular polymer; Therapy; Imaging

Citation: Zhao, M. Z.; Cheng, D. B.; Shang, Z. R.; Wang, L.; Qiao, Z. Y.; Zhang, J. P.; Wang, H. An "*In Vivo* Self-assembly" Strategy for Constructing Superstructures for Biomedical Applications. Chinese J. Polym. Sci. 2018, 36(10), 1103–1113.

INTRODUCTION

Supramolecular chemistry is a highly interdisciplinary domain of science covering chemistry, physics, and biology, which focuses on the ionic/molecular recognition and the assembly of their components through noncovalent interactions[1–5]. Due to the unique well-organized structure induced by noncovalent interactions, the supramolecular system has attracted wide attention in interdisciplinary fields including molecular devices[6], liquid crystal[7], photocatalysis[8] and biomedicine[9]. With the development of nanotechnology, supramolecular chemistry has seeped into self-assembled nanomaterials, such as polymeric nanoparticles[10], liposomes[11], carbon nanomaterials[12] and metallic nanomaterials[13], which plays a vital role in disease diagnostics and therapeutics. The self-assembled nanomaterials could protect the therapeutics from the reticuloendothelial system (RES) and deliver the therapeutics to targeted sites through targeting effect as the result of their special hydrophilic surface, controllable structure and suitable nanometer size[14–18]. Ling and coworkers reported a pH-sensitive nanoassembly which could respond to the tumor micro-environment to realize targeted and enhanced tumor theranostics[19].

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Received May 18, 2018; Accepted June 12, 2018; Published online July 11, 2018

Currently, inspired by ubiquitous biofunctional self-assembly in organisms, constructing functional supramolecules under specific environment could be a promising biotechnique for regulation or imitation of organisms. Xu and coworkers have reported a pioneering work that demonstrated the enzyme-triggered self-assembly of small molecules[20]. Furthermore, the intracellular and pericellular self-assembly have been employed for inhibiting cell growth or overcoming the multidrug resistance[21–23]. In view of the *in vivo* self-assembly enhanced biological function, our group has reported the assembly-induced retention (AIR) effect by the pathologically driven self-assembled nanostructures, which not only enhances molecular retention efficiency but increases the selectivity[24]. In this review, we will introduce various strategies of "*in vivo* self-assembly" and recent representative works of our group about "*in vivo* self-assembled" nanomaterials in disease diagnostics and therapeutics.

STRATEGIES OF "*IN VIVO* SELF-ASSEMBLY"

Up to now, the reported "*in vivo* self-assembled" nanomaterials are mostly based on small peptides (FF), chlorophyll derivative and aggregatable polymer, which must have good biocompatibility and self-organized properties (Fig. 1). Although there are various types of *in vivo* self-assembled nanomaterials, the strategies of "*in vivo* self-assembly" could be summarized as concentration-induced "*in vivo* self-assembly", stimuli-induced "*in vivo* self-

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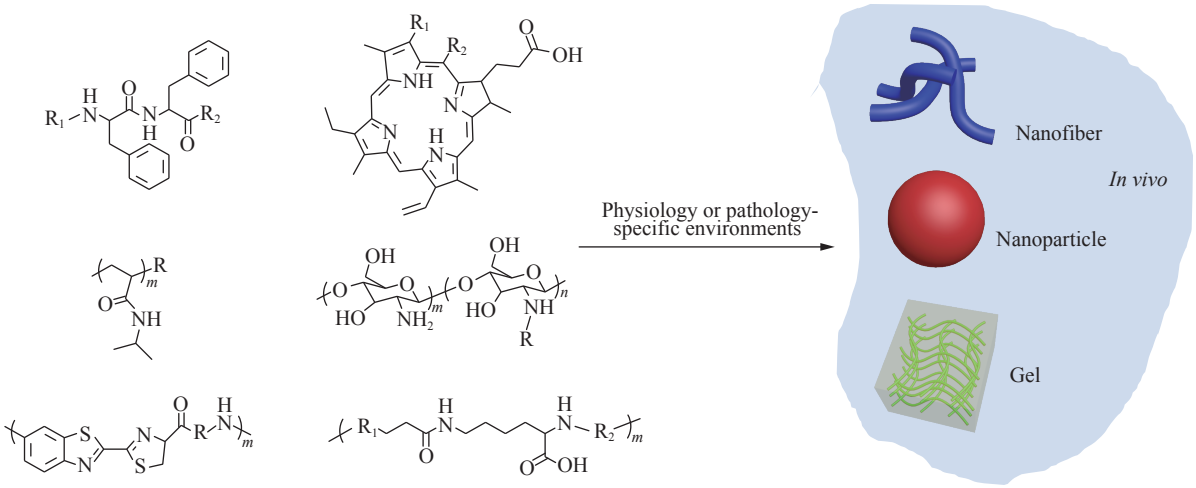


Fig. 1 Molecular structures of reported "*in vivo* self-assembled" nanomaterials and the morphology of assembly

assembly" and condensation-induced "*in vivo* self-assembly".

Concentration-induced "*In Vivo* Self-assembly"

The essence of self-assembly is an equilibrium process between individual units and their aggregates. Self-assemblies can be formed when the concentration of molecules exceeds the critical aggregation concentration (CAC). Unlike water, due to the chemical complexity of *vivo* environment, the concentration of molecules should be higher than CAC for "*in vivo* self-assembly". Thus, the organelle-localization through the targeting effect could rapidly raise the local concentration of molecules, which could be a promising strategy to induce self-assembly *in vivo*. Ryu *et al.*[25] developed mitochondria localization induced supramolecular self-assembly system. Small molecules (Mito-FF), which consist of diphenylalanine and triphenylphosphine (TPP), readily reached to the mitochondria through targeting moiety of TPP. Considering highly negative mitochondrial membrane potential of cancer cells, Mito-FF favorably accumulated in mitochondria, and then self-assembled inside the mitochondria as a result of increased local concentration. The accumulation of molecules inside mitochondria was 500–1000 times higher than that of extracellular space (Fig. 2). The stiff Mito-FF fibers destroyed the mitochondrial membrane, which eventually induced cancer cell apoptosis. The concentration-induced self-assembly offers a new avenue in developing "*in vivo* self-assembly" nanomaterials.

Stimuli-induced "*In Vivo* Self-assembly"

Upon the stimuli such as pH, glutathione reduction, enzyme or metal ions, some water-soluble small molecules could self-assemble and construct nanostructure inside cells or on cell surfaces. Based on the intracellular alkaline phosphatase

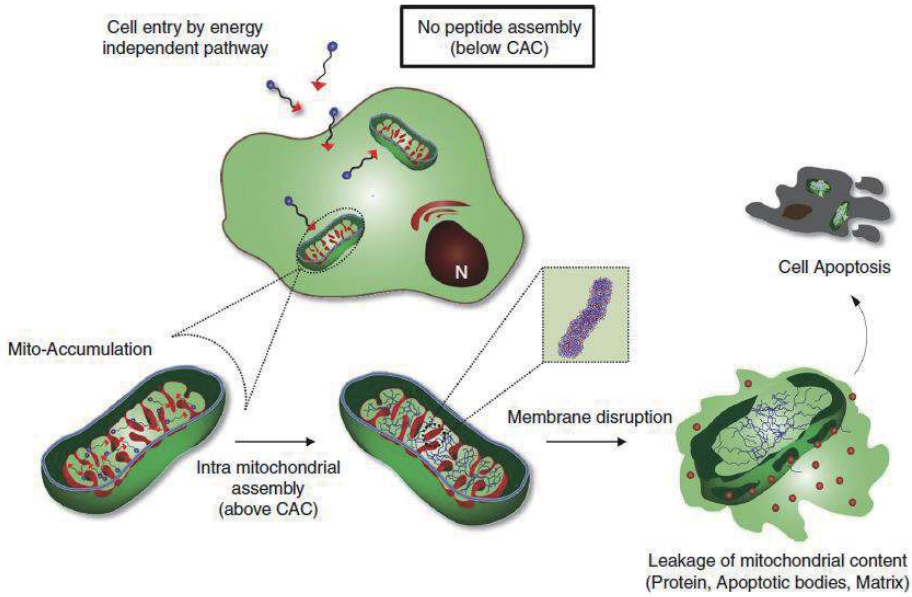


Fig. 2 Intra-mitochondrial assembly of Mito-FF. The increased mitochondrial membrane potential of cancer cells led to high mitochondrial accumulation of Mito-FF, resulting in self-assembly into fibrils. The intra-mitochondrial fibrils could induce cellular apoptosis. (Reproduced with permission from Ref. [25]; Copyright (2017) Nature)

https://doi.org/10.1007/s10118-018-2170-3

that catalyzes the dephosphorylation of tyrosine phosphate residue, Xu *et al*.[26] designed a series of hydrophilic small peptide derivatives which could self-assemble into nanofibers in live cells (Fig. 3). Under enzyme regulation, the molecular structure of precursor was changed from soluble hydrophilic to self-assembling units, resulting in the formation of ordered structure in intracellular or pericellular space. Thus, the departure of hydrophilic motif could be an effective method to realize the "*in vivo* self-assembly" of small molecules.

Except small molecules, polymers could also form nanoassembly under *in vivo* stimuli. Our group demonstrated a thermo-controlled strategy for realizing *in situ* conformational transition of polymer-peptide conjugates on cellular membranes, which could manipulate and monitor Human epidermal growth factor receptor-2 (HER2) clustering[27].

Besides, a series of polymer-peptide conjugates based on thermosensitive polymer backbone and cleavable hydrophilic peptide side chains had been synthesized[28], and the specific responsiveness to endogenous stimuli and controllable construction of uniform nanostructures *in vivo* had been verified (Fig. 4). The thermosensitive polymers with physiological temperature-induced coil/globule transition feature exhibited promising application in constructing functional supramolecules *in vivo*.

Nanoparticles have received significant attention in drug delivery system, and it could deliver the drugs or probes to disease site. Compared with nanoparticles, nanofibers might have different photo-physical properties, multivalent binding ability and size dimension. Ling and coworkers[29] demonstrated the elongated structure of nanofibers was efficiently taken up and retained by tumor, and exhibited a high CT contrast effect. Under the physiological and pathological condition, the physicochemical property of nanoparticles could be changed. Nanoparticles became unstable, and then reassembled into nanofibers. Chen and co-workers[30] reported the overexpressed phosphatase of cancer cells could let the indocyanine green (ICG) and micelles coassemble into nanofibers, which enhanced the tumor accumulation and retention of ICG. Meanwhile, our group has reported the metal ion mediated structural evolution of peptide-based superstructures on specific cancer cell faces[31]. Under the stimulus of Ca^{2+} in integrin $\alpha_v\beta_3$, the nanoparticle with RGD peptide on the cell surface could reassemble into nanofibers *in vivo* (Fig. 5).

Condensation-induced "*In Vivo* Self-assembly"

The condensation of molecules can form more hydrophobic oligomers or polymers, thus facilitate the process of "*in vivo* self-assembly". For example, Rao and co-workers[32] employed condensation reaction between 2-cyanobenzothiazole (CBT) and D-cysteine upon pH adjustment, reduction or enzyme cleavage for constructing supramolecular assembly in cells. Meanwhile, the intracellular self-assembled process was successfully applied to image the activity and localization of protease. Recently, our group have reported the intracellular enzyme-catalyzed polymerization for *in situ* controllable construction of nanostructures[33]. Through the subtle design of small molecules, the intracellular synthesized polypeptide exhibited diverse physiochemical properties and phase transition behaviors. The resulting 3D gel-like elastin-like polypeptides with volume phase transition property at 37 °C

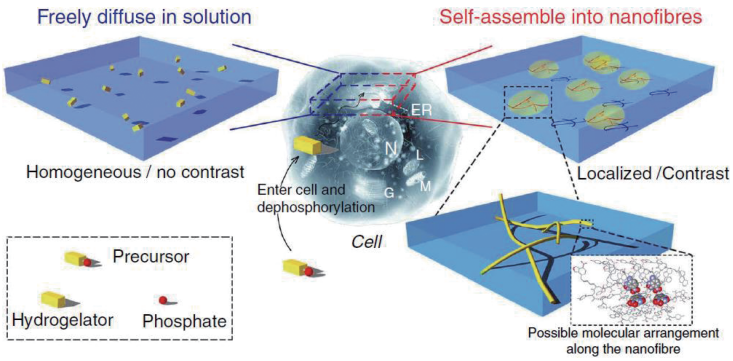


Fig. 3 Principle of imaging enzyme-triggered supramolecular self-assembly inside cells. The small molecules could self-assemble to form nanofibers under the enzyme inside the cell. ER, endoplasmic reticulum; G, Golgi apparatus; L, lysosome; M, mitochondria; N, nucleus. (Reproduced with permission from Ref. [26]; Copyright (2012) Nature)

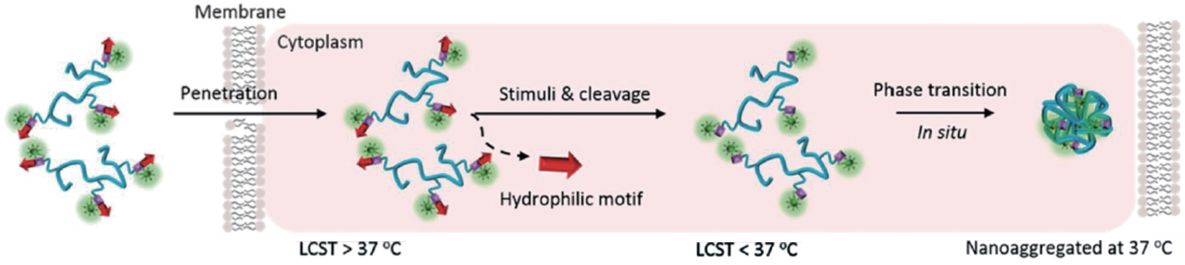


Fig. 4 Schematic representation of endogenous stimuli-induced aggregation of polymer-peptide conjugates (Reproduced with permission from Ref. [28]; Copyright (2017) American Chemical Society)

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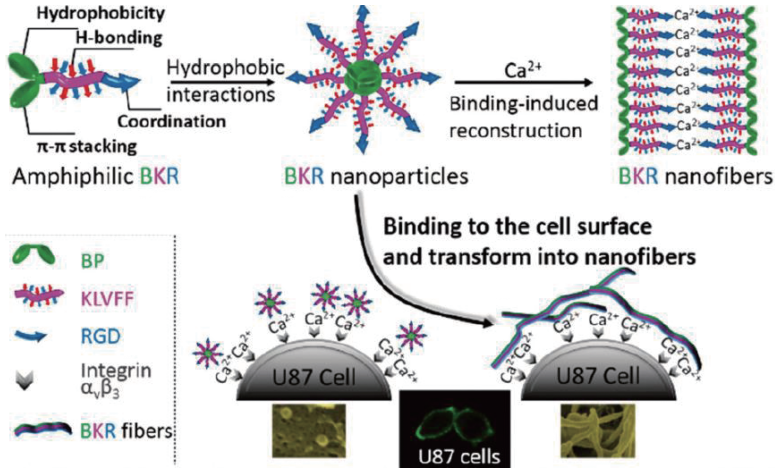


Fig. 5 Schematic representation of metal ion binding induced morphology transformation of BKR nanoparticles to nanofibers in solution and on the cell surface (Reproduced with permission from Ref. [31]; Copyright (2016) The Royal Society of Chemistry)

showed significant cytotoxicity, which could potentially be used for drug-free cancer therapy. This work demonstrates that small molecule peptide units can polymerize with high performance and self-assemble into topologically controlled nanostructures. (Fig. 6)

BIOMEDICAL APPLICATION OF "*IN VIVO* SELF-ASSEMBLY"

In biological systems, the natural self-assemblies are pervasive and play a critical part, such as the cell membrane which is constructed by self-assemblies of the lipid bilayer. To understand and imitate the natural self-assemblies, multiple efforts, such as artificial design of nanoassembly, have been devoted. Beyond the biological function with natural self-assemblies, the artificially designed self-assembled nanoassembly *in vivo* could also have different or even better biological function. Obviously, the nanostructure of molecules significantly affects their pharmacokinetic (PK) and pharmacodynamic (PD) properties *in vivo*; the formation of self-assembly in targeted site could be a promising strategy for disease theranostic[34]. Based on the reported AIR effect, our group has demonstrated various biomedical applications of "*in vivo* self-assembly".

Tumor Imaging and Inhibition

The bioavailability of drugs or probes in biomedical applications significantly affects their performance. Due to the complex tumor microenvironment, many strategies have been developed to enhance biological properties of drugs and probes by promoting the tumor accumulation, such as enhanced permeability and retention (EPR) effect, long-term blood circulation, passive targeting mechanisms and so on. Up to now, constructing the nanoassembly at tumor site has been demonstrated to be a rising strategy for effectively

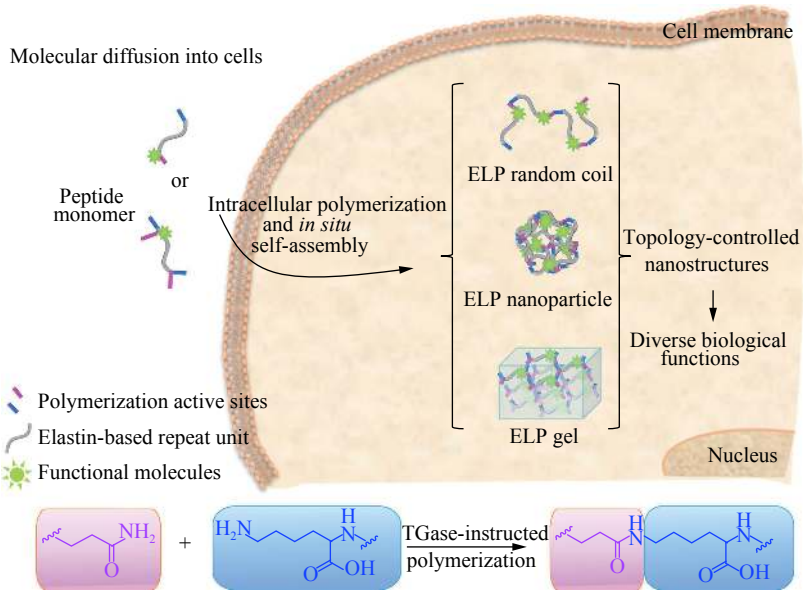


Fig. 6 Intracellular polymerization of peptide monomers and *in situ* self-assembly of the polypeptide (Reproduced with permission from Ref. [33]; Copyright (2017) Nature)

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enhancing the retention of drugs or probes, which has great potential for tumor bioimaging and inhibition.

Tumor imaging

Tumor imaging is a technique to visualize, characterize and measure tumor at cellular and molecular levels in living organisms, which is indispensable in tumor diagnostics and therapy[35]. Due to the effective retention, the *in situ* formation of assembly at tumor site could increase the signal-to-background ratio and specificity of imaging agents. Rao and co-workers[36] introduced a photoacoustic (PA) probe, which could monitor the proteolytic activity of furin in tumor site *via* constructing nanostructures. The oligomerizable probe produced a significantly higher PA signal in tumor overexpressing furin, and had the potential for highly specific and early detection of tumor. In further research, Rao and co-workers employed the *in situ* self-assembly of small molecules for successfully imaging the caspase-3/7 activity *in vitro* and *in vivo*, and all the fluorescence imaging, magnetic resonance imaging (MRI) and position emission tomography imaging (PET) techniques were achieved for long-term and high resolution imaging[37–39].

For better understanding the biological performance of self-assembled nanostructures, our group[24] subtly designed a responsive small molecule compound 1 by combining targeting peptide (RGD), responsive peptide (PLGVRG) and photosensitizers (purpurin 18), which could evaluate the retention effect by monitoring long-term PA imaging signals (Fig. 7a). The hydrophilic small molecule could reach the tumor site through the interaction between $\alpha_v\beta_3$ and RGD, and then under the gelatinase that selectively cut the PLGVRG liker, the residue of purpurin 18 with enhanced hydrophobicity and reduced steric hindrance could self-assemble into nanofibers (Figs. 7b and 7c). Compared to nonresponsive group that could not assemble into nanofibers, the designed responsive small molecule precursor showed the maximum of sevenfold higher retention concentration at 10 h post-injection. Moreover, the half retention time ($\tau_{1/2}$) of responsive small molecule precursor was up to 24 h and $\tau_{1/2}$ of nonresponsive group was only 4 h. The self-assembled fibrous nanostructures in tumor exhibited prolonged retention time, which was attributed to the AIR effect. At last, the constructed nanofibers could be disassembled and excreted out of the solid tumor through slowly hydrolysis of purpurin 18 (Fig. 7d). Similarly, the strategy of "*in vivo* self-assembly" has been employed for real-time and quantitative detection of autophagy, so as to optimize the therapeutic effect under the supervision of the autophagic signals[40].

Tumor therapy

The tumor accumulation of active therapeutic is a crucial

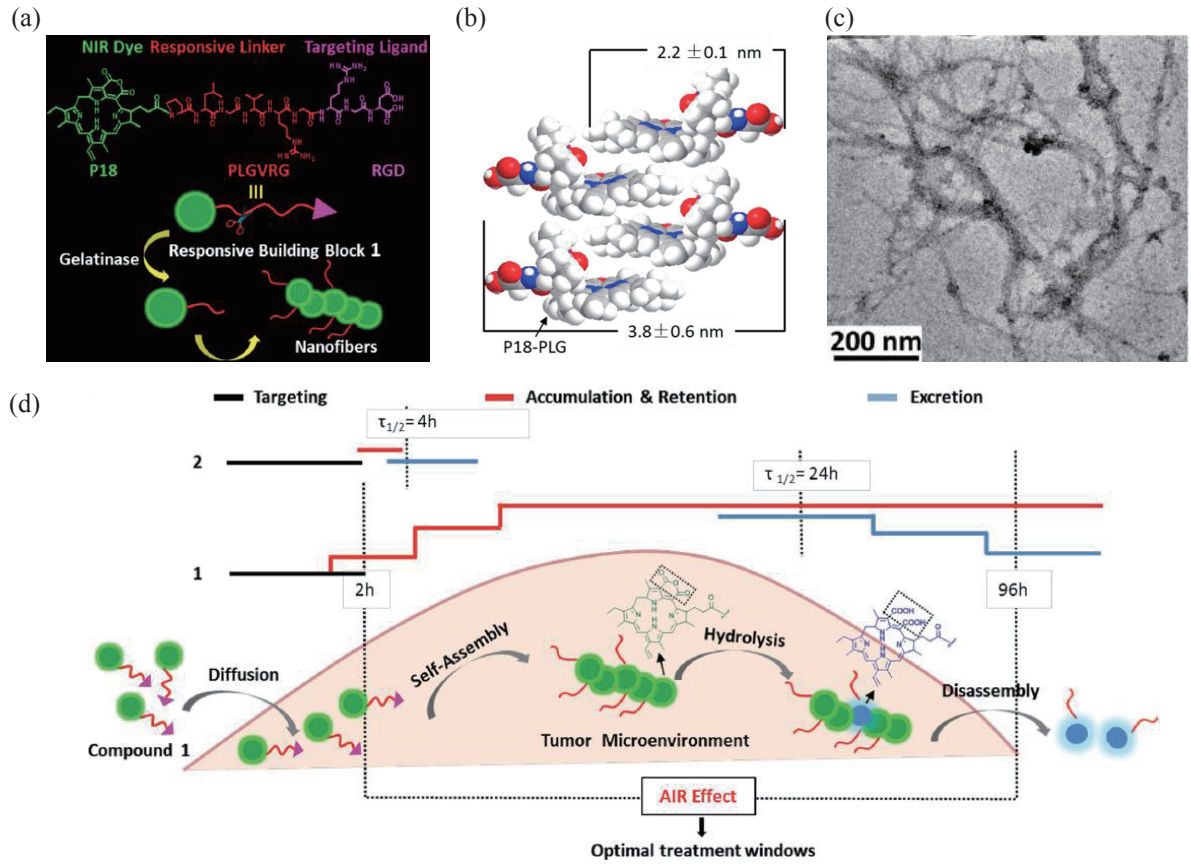


Fig. 7 (a) Molecular structure and the process of assembling molecules compound 1 into nanofibers; (b) Schematic space-filling modes illustrating the self-assembly of molecules; (c) Morphology of compound 1 after incubating with gelatinase in the buffer solution; (d) The schematic illustration of the AIR effect of compound 1 in tumor site compared with the controlled compound (Reproduced with permission form Ref. [24]; Copyright (2015) Wiley-VCH)

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indicator for systemic administration. Although some small molecules as therapeutics could reach the tumor site though targeting effect, they are easy to be excreted from tumor site, which could reduce the bioavailability of therapeutics and give rise to side effects[41]. Intriguingly, larger self-assembly from small molecules can be trapped in tumor tissue and exhibit long-time retention in tumor site. Considering that the efficacy of drugs could be reduced in assembling form, Xu and co-workers[42] have properly designed and synthesized a precursor for producing a taxol assembly without compromising activity of the taxol. The intracellular enzymatic self-assembly provided a powerful method to create drug assembly, which could be employed for tumor targeted therapy. Unlike the chemotherapy drugs, the photothermal and photodynamic agents could work well or even better in the form of assembly. Our group[24] took advantage of assembly of purpurin 18 from small molecules for enhanced tumor photothermal therapeutic efficacy, and the results showed great potential in tumor therapy.

Nanoparticles have been actively developed for therapeutic agent delivery due to their merits such as protecting the hydrophobic therapeutic agents from the aqueous environment, prolonged circulation time and accumulation at tumor sites through enhanced permeability and retention (EPR) effect[21]. To achieve a longer blood circulation and better tumor accumulation, the generally accepted particle size of nanoparticles is about 10–100 nm. Intuitively, small nanoparticles have a better diffusivity, which is required for deep tumor penetration, than large particles in tumor tissue. Unfortunately, small nanoparticles, as well as small molecules, cannot be retained in tumor tissue effectively because they are able to re-enter the bloodstream[41]. Thus, transformation from small nanoparticles to large microparticles or fibers in tumor sites can be an optimal solution to achieve deep penetration and enhance tumor retention. Gianneschi *et al.*[43] generated nanoparticles through pacltitaxel-conjugated diblock copolymers. The hydrophilic shell of peptide sequence GPLGLAGGERDG could be recognized and cleaved by matrix metalloproteinases, then spherical nanoparticles with 20 nm in diameter could transform into micrometer scale assemblies. These larger drug assemblies could slowly release the pacltitaxel *via* hydrolysis within the tumor tissue, which exhibited enhanced safety and efficacy of chemotherapy. A similar work by Gianneschi group also demonstrated that the transition from small nanoparticles to large assemblies is capable of achieving prolonged retention in acute myocardial infarction[44]. This unique approach constitutes a minimally invasive method for delivery of a material scaffold to acutely infarcted myocardium.

Interestingly, inspired by a pathological evidence that small molecules are readily to intercalate into Aβ fibers for detection of Alzheimer disease, our group[45] *in situ* constructed peptide-based self-assembled nest-like hosts as noninvasive implants in tumor region for homing theranostic agents. The peptide derivative consisted of four motifs (Fig. 8a): hydrophobic and aromatic bis-pyrene motif (BP), hydrogen-bonding peptide KLVFF originated from Aβ, hydrophilic poly(ethylene glycol) (PEG) chain and pH-responsive peptide motif (His_6). BP-KLVFF-His_6-PEG could form nanoparticles ((39 ± 9) nm) in neutral condition (pH 7.4) and transform into nanofibers ((6.9 ± 1.6) nm) in acidic buffer (pH 6.5) due to the altered hydrophilic/lipophilic balance (Figs. 8b and 8c). The diameter of nanofibers was

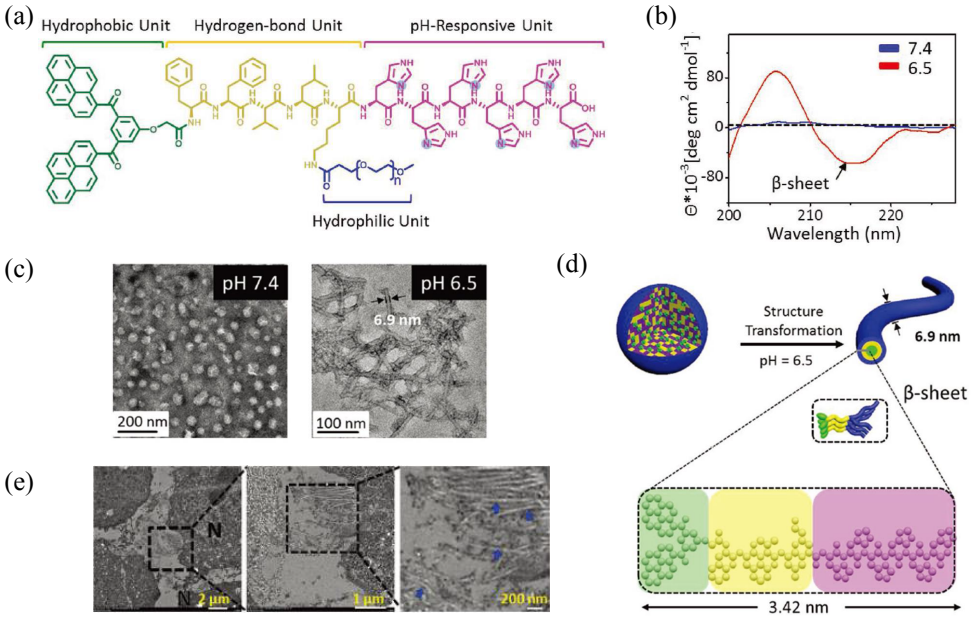


Fig. 8 (a) Molecular structure of peptide derivative with building blocks BP, KLVFF, His_6, and PEG1000 motif; (b) CD spectral characterization of transformation process with the β-sheet structure formation; (c) The BP-KLVFF-His_6-PEG maintained the particulate morphology at pH 7.4 and transformed from nanoparticles to nanofibers observed by TEM at pH 6.5 under 37 °C after 2 h culture; (d) Schematic illustration of morphology transformation process from nanoparticles to nanofibers with decreased pH; (e) The TEM images of tumor tissue slices of xenografted mice (Reproduced with permission from Ref. [45]; Copyright (2017) Wiley-VCH)

https://doi.org/10.1007/s10118-018-2170-3

consistent with the length of two molecules (Fig. 8d). This BP-KLVFF-His6-PEG initially self-assembled into nanoparticles, and then, after the nanoparticles were injected into tumor-bearing mice, the nanoparticles could accumulate in the tumor site, and transform into nanofibers with β-sheet structures due to tumor-microenvironment acidity (Fig. 8e). Compared with peritumoral and intracavitary injection, the fluorescence intensity of tumor by intravenous injection of nanoparticles was stronger and the whole tumor was lit up. Nanofibers in tumor showed a long retention time up to 96 h, and could effectively bind and enrich the subsequently free molecules (Nile red, indocyanine green and doxorubicin). At 96 h, the fluorescence intensity of accumulated NR in tumor could reach 7.5-fold than that of free NR-treated groups. The noninvasive delivery system was validated to effectively improve the bioavailability of small molecules and enhance treatment efficacy for tumors.

It is reported that the artificial nanostructures can affect the cellular behavior and cause cell death[20]. Wells and co-workers[46] reported a kind of nanofibrils formed from small molecules which would bind to procaspase-3, then activated its transformation into caspase-3 and initiated the apoptosis cascade. Encouraged by this study, Xu and co-workers evaluated the cytotoxicity of the diphenylalanine assembly and suggested that this assembly could disrupt the dynamics of microtubules causing apoptosis of cells[47]. Recently, similar researches about cytotoxicity of self-assemblies to cancer cells, especially drug resistant cancer cells, have been developed and showed great potential in tumor therapy[48–50]. Our group *in situ* constructed an artificial extracellular matrix (AECM) based on laminin-mimic peptide for inhibition of tumor invasion and metastasis[51]. This laminin-mimic peptide (1-NPs) was composed of bis-pyrene (BP), KLVFF peptide and Y-type RGD-YIGSR motif (Fig. 9a). 1-NPs was supposed to form nanoparticles with the hydrophobic BP as the core and hydrophilic peptide as outer layer. Then, the spherical structure of 1-NPs could transform into fibrils when incubated with $CaCl_2$ aqueous solution (Fig. 9b). 1-NPs treated MDA-MB-231 cells showed green fluorescence on cell surfaces, probably due to the *in situ* transformation of 1-NPs, which could inhibit the internalization of 1-NPs (Figs. 9c and 9d). Furthermore, the fibrous network surrounded cells were observed because of the transformation of 1-NPs on cell surfaces (Figs. 9e and 9f). The as-prepared nanoparticles were intravenously injected into tumor-bearing mice, then transformed into nanofibers around the tumor upon binding to receptors on tumor cell surfaces and entwined to form AECM. This biomimic assembly constructed *in vivo* provided an effective drug-free strategy for inhibition of tumor invasion and metastasis.

Bacterial Infection Diagnosis and Therapy

Pathogenic infections remain a threat to public health due to the drug resistance raised from the abuse of antibiotics[52]. There is an urgent need for bacterial infection diagnosis and therapy for the purpose of early detection and less resistance. Similar to tumor microenvironment, the bacterial infectious microenvironment also differs from normal tissue, such as the enzyme activity or the concentration of reactive oxygen species[53]. In terms of the different environments between infection sites and normal tissue, constructing assemblies in infection sites has been developed and exhibited promising performance for bacterial infection diagnosis and therapy.

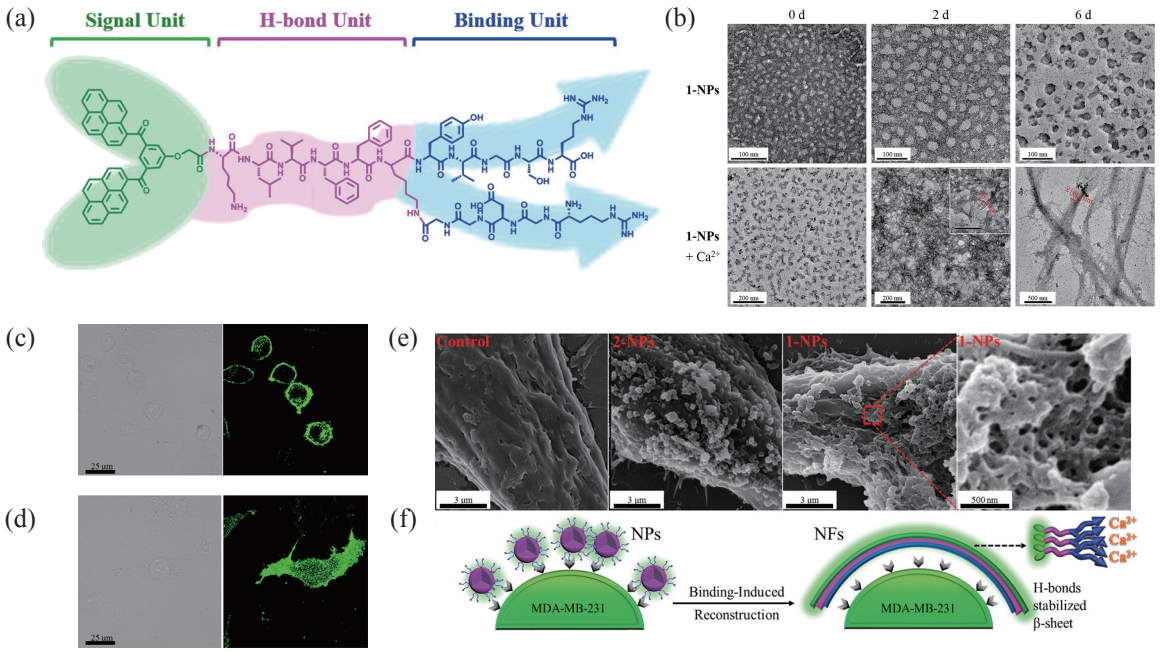


Fig. 9 (a) Molecular structure and schematic illustration of BP-KLVFF-GGDGR-YIGSR; (b) TEM images for the morphologies of 1-NPs nanoaggregates CLSM images of MDA-MB-231 cells incubated with (c) 1-NPs and (d) controlled peptide for 2 h; (e) SEM images of cell surfaces of MDA-MB-231 treated with 1-NPs and control; (f) Schematic illustration of ligand-receptor interaction induced structural transformation of 1-NPs to nanofibers on cell surfaces (Reproduced with permission from Ref. [51]; Copyright (2017) American Chemical Society)

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Although various strategies for early-stage bacterial detection exist, most of them cannot be used *in vivo* since they suffer from low sensitivity and specificity. To solve this problem, our group presented a new photoacoustic contrast agent for specific and sensitive imaging of bacterial infection *in vivo*[54]. The PA contrast agent was composed of pyropheophorbide-*α* (Ppa) as a signaling molecule, peptides PLGVRG as an enzyme-responsive linker, and vancomycin (Van) as a targeting ligand (Fig. 10a). After Ppa-PLGVRG-Van was cleaved by gelatinase, Ppa-PLG self-aggregated in aqueous solution owning to its increased hydrophobicity and reduced spatial hindrance (Figs. 10b and 10c). The agent could reach to the infection sites, and then respond to gelatinase to form nanofibers, which were confirmed by the tissue-mimicking phantoms with infection of various species of bacteria. Four species of bacteria (*S. aureus*, *E. coli*, *S. epidermidis* or *P. vulgaris*) and sterile myositis were investigated and the agent demonstrated the high specificity to *S. aureus* (Gram-positive, gelatinase-positive) (Fig. 10d). Interestingly, after the agent was intravenously injected into mice, PA signals at bacterially-infected sites were significantly higher than control sites up to 12 and 24 h. The enhanced PA signals may be attributed to the high retention of self-assemblies at the infected sites (Fig. 10e). Meanwhile, the detection limit of the agent was declined, about 2 orders of magnitude lower than the previous reports.

The antibiotic is of irreplaceable significance in defending bacterial infection. However, the severe antibiotic resistance reduced the lifetime of new antibiotic[55, 56]. Numerous antibiotic alternatives such as photothermal agents, photodynamic agents, cationic polymers and antimicrobial peptides have been exploited[57]. Recently, the strategy of "*in vivo* self-assembly" worked promisingly in bacterial infection therapy. Xu and co-workers[58] first reported the enzymatic self-assembly inside *E. coli*, and the resulting intracellular self-assembly could inhibit the bacterial growth effectively. Another work of bacterial surface-induced self-assembly was also applied for inhibition of bacteria. Besides the AIR effect, our group demonstrated an "on-site transformation" strategy for designing antibacterial agents with enhanced binding capability and killing efficacy[59]. A chitosan-peptide conjugate (CPC), which contained chitosan backbone, enzyme-cleavable peptide (GPLGVRGC) with a poly(ethylene glycol) (PEG) terminal and antibacterial peptide CGGG(KLAKLAK)$_2$ (KLAK), was synthesized and could self-assemble into nanoparticles in water (Fig. 11a). Once CPC nanoparticles arrived at the infection site, the protecting PEG layers were shed off in the presence of gelatinase, resulting in disruption of hydrophobic/hydrophilic balance and transition into fibrous structures *via*

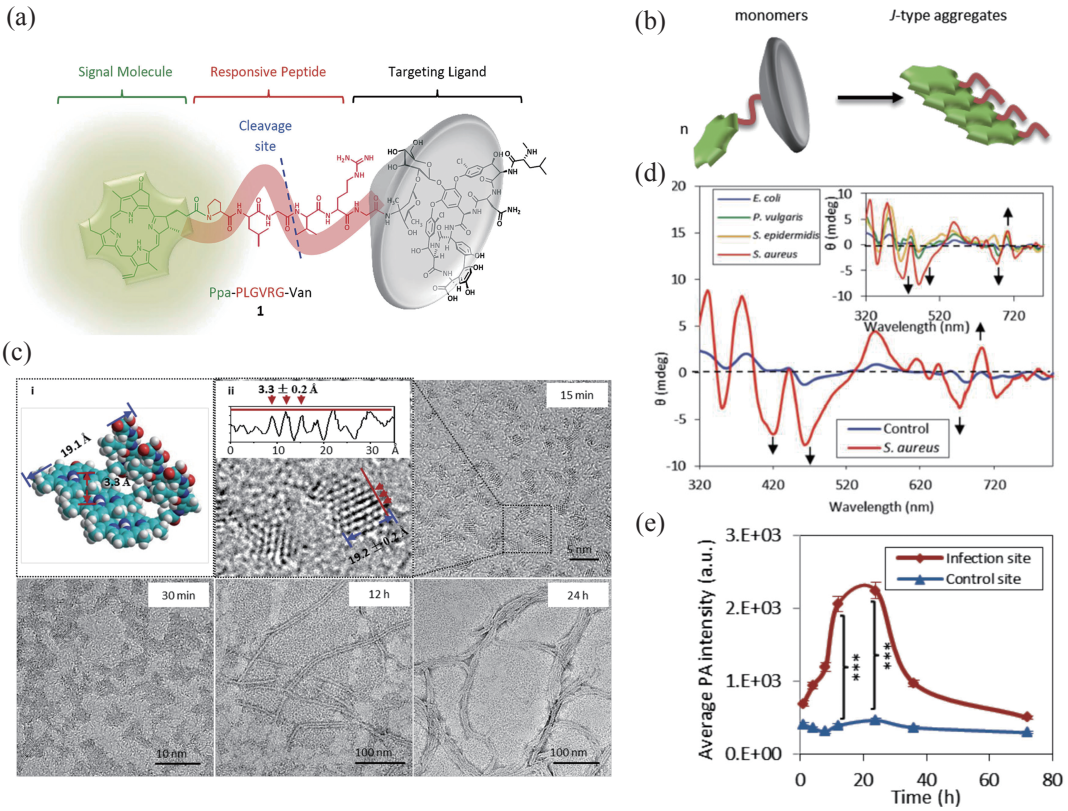


Fig. 10 (a) Molecular structure and schematic illustration of Ppa-PLGVRG-Van; (b) Scheme showing monomers and aggregates; (c) Schematic space-filling models illustrating the self-assembly of Ppa-PLG (i), high-resolution TEM image of aggregates after 15 min (ii), and TEM images of aggregates of molecule obtained after 15 min, 30 min, 12 h, and 24 h; (d) CD spectra of Ppa-PLGVRG-Van after exposure to phantoms seeded with *S. aureus*, *E. coli*, *S. epidermidis*, *P. vulgaris* or without bacterial seeding. Arrows indicate the main spectral changes in the *S. aureus* sample compared to the control; (e) Average PA signal of the control site and infected site over a span of 72 h after injection with Ppa-PLGVRG-Van (Reproduced with permission from Ref. [54]; Copyright (2016) Wiley-VCH)

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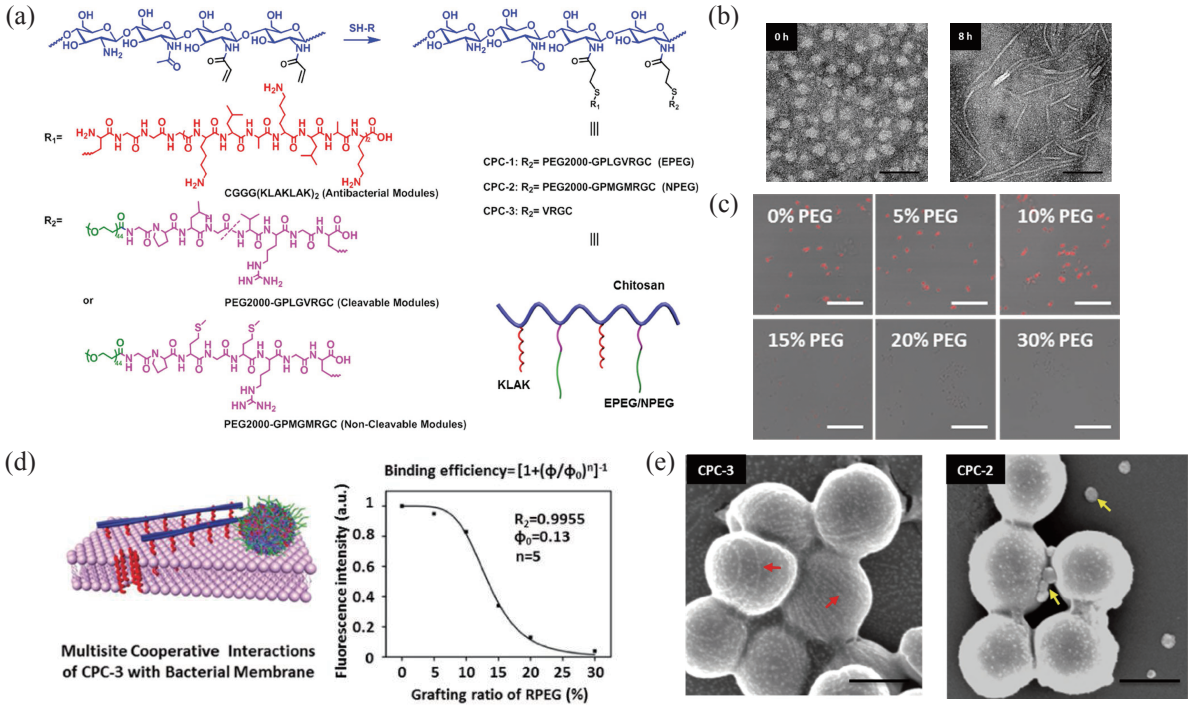


Fig. 11 (a) Synthetic scheme for preparation of CPCs. R1 represented the antibacterial peptide KLAK; the conjugates were named as CPC-1 and CPC-2 when R2 represented EPEG (gelatinase-cleavable peptide (GPLGVRGC) with PEG2000 terminal) and NPEG (control peptide (GPMGMRGC) with PEG2000 terminal), respectively. The synthetic CPC-3 was used for mimicking the residue of CPC-1 cleaved by gelatinase; (b) TEM images of CPC-1 nanoparticles after immersed in gelatinase (10 μg·mL^{-1}) Tris buffer solution (pH 7.4) with 0 and 8 h. Scale bars, 100 nm; (c) Fluorescent images of CPC-1 with varying PEG grafting ratios incubated with *S. aureus* for 1 h in static culture. The fluorescence intensity of bacteria inversely proportional to EPEG grafting ratio of CPC-1; (d) Schematic cartoon of CPC-3 binding model toward bacteria; The Hill exponent, n = 5, suggested strongly multisite binding of fibrous CPC-1 to the bacteria surface; (e) SEM images of *S. aureus* incubated with spherical CPC-2 and fibrous CPC-3 for 1 h. Yellow and red arrows indicated nanoparticles and nanofibers, respectively. Scale bars, 500 nm (Reproduced with permission from Ref. [59]; Copyright (2017) Wiley-VCH)

chain-chain interaction of chitosans (Fig. 11b). The *S. aureus* incubated with varying grafting ratios of PEG showed evident increase in mean bacteria fluorescence with low grafting ratios, the effective Hill exponent of n = 5, suggesting that multisite attachment occurred between bacteria and fibrous CPCs (Figs. 11c and 11d). Furthermore, the binding model of CPC-3 was visually observed using SEM (Fig. 11e). Compared with morphology-unchanging CPC-2, the transformable CPC-1 not only exhibited up to four-time prolonged retention time in infection sites, but remarkably elevated the multisite cooperative electrostatic interaction between nanomaterials and bacteria. Thus the "on-site transformation" strategy with increasing binding, effective accumulation and prolonged retention possesses great potential for treatment of bacterial infection.

CONCLUSIONS AND PERSPECTIVE

This article briefly summarizes the strategy as well as some recent works of "*in vivo* self-assembly". Based on the AIR effect, constructing supramolecules *in vivo* could enhance the specificity and bioavailability of molecules, which presents an improvement of biomedical application. However, the research about "*in vivo* self-assembly" is still in an early stage, and it is still challenging to be advanced due to the staggering complexity of *vivo* environment. Despite that various molecules can form assemblies in water, little occurs in live animals. The process and location of "*in vivo* self-assembly" occurrence is also difficult to control, which will positively impact the biological function of assemblies. In general, precursors should be stable before reaching to the targeted site, and then form assembly *in situ*. Thus the physiology or pathology-specific environment may be the desirability for controlled self-assembly *in vivo*.

The ultimate goal of "*in vivo* self-assembly" is: (i) illustrating the transition process from molecules to assemblies and the symbiotic relationship between assemblies and organisms; (ii) *in situ* constructing functional materials for regulation or imitation of organisms and their clinical applications. As with proteins, artificially designed nanoassemblies may present a specific bioactivity with defined three dimensional structure, which can be used for disease diagnostics and therapeutics. It should be noted that the practical application of constructing nanomaterials *in vivo* is still farfetched, and more investigations into this system will be desirable and rewardable. We hold the belief that "*in vivo* self-assembly" nanosystem will become a new avenue of promising nanomedicine platforms for public healthcare.

https://doi.org/10.1007/s10118-018-2170-3

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (Nos. 21704020, 21374026, and 51573032) and the National Science Fund for Distinguished Young Scholars (No. 51725302). Dr. Dong-Bing Cheng acknowledges financial support from the China Postdoctoral Science Foundation (No. 2017M620707).

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https://doi.org/10.1007/s10118-018-2170-3