In Vivo Self-Assembly of Polypeptide-Based Nanomaterials

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36.1 Introduction

Polypeptide is a chain consisting of no more than 50 amino acid monomers linked by peptide bonds. Compared with proteins, peptides lack complex stereochemical structures but perform functions vital to biological systems. With non-covalent interactions, peptides can self-assemble into two-dimensional or three-dimensional structures.

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The wide existence of peptides in human bodies makes them have intrinsically biological functions, such as bioactive, biodegradable, and biocompatible. Those features have been deemed to be the building blocks of biological materials [1]. During the half-century of research and development, thousands of peptide sequences have been applied in the field of biopharmaceutics.

They can increase the targeting of drug delivery, promote the half time of small molecules in circle system, express as a therapeutic drug, etc. Different functional short peptides can combine into a long peptide chain and express a tandem function [2, 3] (Fig. 1).

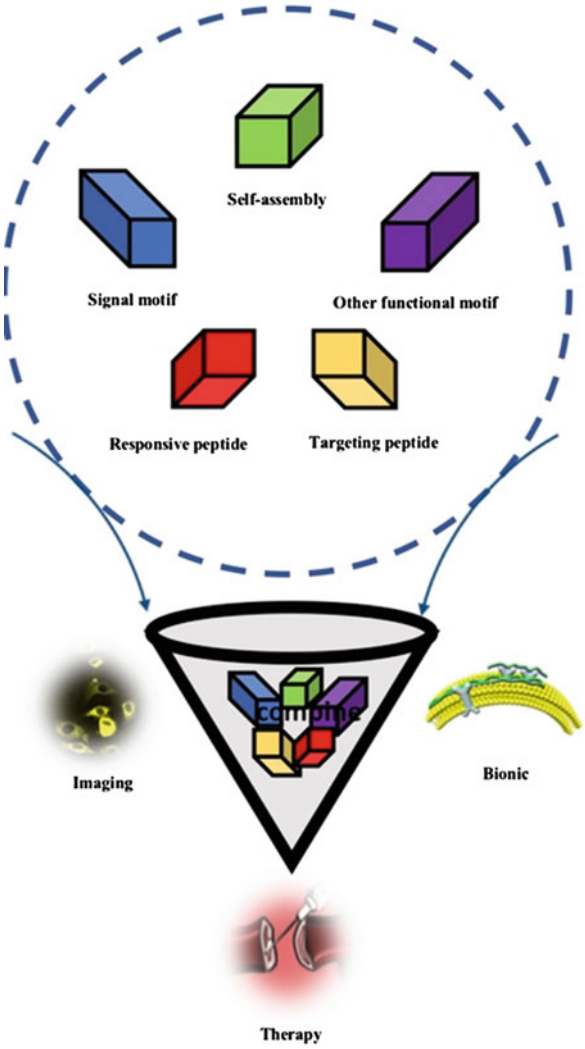

Fig. 1 The principle of designing in situ self-assembled peptide-based nanomaterials and their applications

Another highlight research about peptides is their superstructure. From now on, several kinds of peptide-based structures, such as nanotube, nanofibril, nanoparticle, nanowire, etc., are designed and applied to perform special functions [4, 5]. The driving forces, mainly non-covalent interaction, include hydrogen bonding [6], $\pi - \pi$ stacking interactions [7], hydrophobic-hydrophilic interactions [5], and ionic bonding [8]. These highly ordered peptide nanostructures can be used in bioimaging, cancer therapy, tissue engineering, antibacterial, and regenerative medicine field [1]. For example, Wang and his co-workers utilized nanofibrils to enhance bioactive molecule retention at disease sites [9]. Joel H. Collier and his co-workers used Q11 self-assembling peptides to co-assemble into a "β tail" structure to deliver multiple proteins [10]. Samuel I. Stupp and his co-workers designed a peptide-amphiphile self-assembly induced by pH to make a nanofibril scaffold reminiscent of extracellular matrix [11].

Recently, a concept "in vivo self-assembly" has been proposed. Compared to the in vitro self-assembly, in vivo peptide self-assembly has a structural transformation induced by other in vivo microenvironment factors. This strategy can help small molecules to in situ form nanostructures and at the same time escape biological barrier. Yang and his co-workers utilized phosphatase and glutathione (GSH) to realize tandem self-assembling in liver cancer cells [12]. Wang and his co-workers designed a three-module peptide to realize the morphological transformation from nanoparticles to nanofibrils [2]. The detailed principle and mechanism will be analyzed in section "In Vivo Self-Assembled Peptide-Based Nanomaterials."

In this chapter, we will focus on in vivo self-assembled peptide-based nanomaterials and explain their design principles and applications.

36.2 Functional Peptides and Driving Forces of Self-Assembly

36.2.1 Category of Functional Peptides

As building a tower needs brick, concrete, and rebar, an applied peptide chain should always be made up of different functional peptides. The scientists need to select various functional peptides so that they can realize different aims. In this section, we focus on the "in vivo self-assembly" relative functional peptides and separate them into targeting and responsive peptide self-assemblies (section "Driving Forces of Peptide Self-Assembly" followed by the driving forces of self-assembly) and give a detailed introduction.

36.2.1.1 Targeting Peptides

To some extent, targeting peptides, instead of monoclonal antibodies, can target cell surface ligand receptors. However, antibodies, which essentially belong to a family of proteins, have a large molecular weight. And the cost of production limited its clinical application scale. The binding abilities of peptides give targeting capability to different tissues and lesions. In strategy of "in vivo self-assembly" system, cell

Table 1 Targeting peptide. N.A.: not available

Targeting position	Peptide sequence	Length	Receptors	Refs.
Tumor vasculature	RGD	3	Integrin $\alpha_V\beta_3\alpha_V\beta_5$	[16]
	NGD	3	Aminopeptidase N	[17]
	IFLLWQR	7	Annexin1	[44]
	CGLIIQKNEC	10	Clotted plasma protein	[45]
	CREAK	5	Clotted plasma protein	[46]
Extracellular matrix (ECM)	CRRHWGFEFC	10	MMP-2/9	[19]
	CTTHWGFTLC	10	MMP-2/9	[19]
	CPIEDRPMC	9	$\alpha_5\beta_1$	[47]

surface targeting [2], tumor microenvironment targeting [13], and bacteria-infected location targeting [14] peptides were widely used.

RGD and NGR are the first two targeting peptides shown in the peptide libraries [15]. RGD, a typical peptide to target tumor microenvironment, can bind to integrins $\alpha_V\beta_3$ and $\alpha_V\beta_5$ which specifically exist in tumor blood vessels [16]. Similar to RGD, NGD is also a vital tripeptide to target tumor vasculature, corresponding to another different receptor-aminopeptidase N [17]. In tumor microenvironment, matrix metalloproteinase (MMP) is another overexpressed substance; thus, it can degrade extracellular matrix (ECM) to enable tumor cell metastasis [18]. The peptides CRRHWGFEFC and CRRHWGFEFC have exhibited high affinity for MMP-2 and MMP-9 and can inhibit their enzymatic activities [19]. The peptide TGRAKRRMQYNRR can utilize electrostatic interactions to bind to bacteria membrane, targeting the bacteria-infected region for imaging and treatment [18, 20]. As a fragment of amyloid β (Aβ), the peptide KLVFF and its derivative can combine with Aβ and inhibit Aβ to form β-sheet structure aggregation [21]. Wang and his co-workers used KLVFF to target Aβ entering the cells, and promoting the degradation of Aβ upregulates autophagy [22]. Some other targeting peptides are summarized in Table 1.

36.2.1.2 Responsive Peptides

In nature, some peptides, which have a significant conformational change when simulated by the microenvironment factors, are called responsive peptides. These switch-peptides play an important role in morphology transformation, which is one of the most significant keys of "in vivo self-assembly" strategy. The stimuli factors are pH, temperature, light, enzyme, metal ions, etc. (Table 2).

Zhang and co-workers have reported a peptide chain EAK12-d (AEAEAEAEAKAK) that could transform from a β-sheet to an α-helix structure in response to pH or temperature changes [23]. Elastin-like polypeptides (ELPs) are biopolymers which have a transition temperature (T_t). When below the T_t, they have good solvability in aqueous solution but aggregate beyond the T_t. ELPs are combined by pentapeptide repeat VPGXG where X is another amide acid except for proline. Wang and his co-workers utilized ELP monomers to design topology-

Table 2 Responsive peptides

Stimulations	Peptide sequence	Length	Refs.
pH	AEAEAEAEAKAK	12	[23]
	ADADADADARARARAR	16	[48]
	$H_7K(R_2)_2$	12	[33]
	KIAQLKYKISQLKQ	14	[49]
	EIAQLEYEISQLEQ	14	[49]
	VKVKVKVKVPPTKVKVKVKV	20	[50]
	VKVKVKTKV$_D$PPTKVKVKVKV	20	[50]
Enzyme			
MMP-2	PLGVRG	6	[38]
	GPVGLIGK	8	[51]
	GPLGIAGQ	8	[52]
	PVGLIG	6	[53]
Furin	RVRRCK	6	[51]
MMP-1	GPQGIAGQ	8	[54]
	GPQGIWGQ	8	[54]
	APGL	4	[54]
Caspase-3/7	DEVD	4	[26]
MMP-7	VPLSLTM	7	[55]
	RPLALWRS	8	[56]
MMP-13	PQGLA	5	[57]
MMP-9	FFFFCGLDD	9	[58]
	PVGLIG	6	[53]

controlled nanostructures and in situ self-assembly by both intracellular TGase-catalyzed and temperature response [24].

Enzymes can often trigger responses for responsive peptides. As an important overexpressed enzyme in tumor microenvironments, MMPs are often considered when designing nanostructures. The peptide GPLGIAGQ can be cleaved into GPLG and IAGQ by MMP-2 and PVGLIG into PVG and LIG by MMP-2/MMP-9. Caspase, a kind of protease that is closely related to apoptosis, can recognize and cleave specific peptide chains [25]. For example, DEVD is a short peptide that can be cleaved by caspase-3/caspase-7 [26]. Other responsive peptides are shown in Table 2.

36.2.2 Driving Forces of Peptide Self-Assembly

The application of polypeptides is restricted by poor stability in vivo and short half-life, which is easy to be digested by protease and quickly eliminated in vivo. Self-assembly is considered to be one of the most promising methods to promote the stability of peptide-based nanomaterials through hydrogen bonds, ionic bonds, and π-π interactions.

As precursors of proteins, peptides have well-defined secondary structures, with the most prominent being the α-helix and β-pleated sheet structure. α-Helix structures, with a periodicity of 3.6, are formed through hydrogen bonds between amide backbones [27]. Smith and co-workers designed two complementary leucine-zipper peptides (SAF-p1 and SAF-p2) that can assemble into two-stranded coiled-coil rods [28]. This system can co-assemble to form long and thickened protein fibers about 4.2 nm along the fiber axis. Hydrogen bonding between amino acid backbones can form β-sheets that form parallel or antiparallel structures. The aggregation of which will always resemble nanofibers. The first report of β-sheets identified in proteins was in the early 1950s by Pauling, Corey, and others [29]. The major mechanism of self-assembly is hydrogen bond. KLVFF is a segment of Aβ sequence which is a significant reason of the formation of Alzheimer's disease (AD). The pentapeptide can form nanofibers by hydrogen bonds [21]. Besides, most β-sheet peptide monomers have hydrophobic and hydrophilic sides. For example, multidomain peptides (MDPs) can first form a dimer with hydrophobic interaction in solution and then form nanofibers with a hydrophobic core by the peptide dimer [30]. This kind of peptides can extend their length by adjusting the pH and salt composition of solvent. Stupp's groups have designed a kind of amphipathic peptide which can self-assemble into β-sheet nanostructure [31]. In addition, enough ionic bonds interact with periodic repetition of the hydrophilic surface, and π-π interactions, such as diphenylalanine (FF), are able to further stabilize the β-sheet structure. Inspired by FF, some aromatic compounds were developed to conjugate with peptides and self-assemble by π-π interactions, such as carbobenzyloxy, naphthalene, Fmoc, bis-pyrene (BP), and purpurin 18 (P18) [7]. Some typical self-assembled peptides were summarized in Table 3.

36.3 In Vivo Self-Assembled Peptide-Based Nanomaterials

36.3.1 The Properties of "In Vivo Self-Assembled Peptide-Based Nanomaterial"

The "in vivo self-assembly strategy" indicated that the internal environment factors, such as pH, enzyme, ligand-receptor interaction, etc., stimulate the structure or morphology transformation of peptide-based nanomaterials. The two vital aims to design such nanomaterial are (i) the ability to self-assemble in a target area and remain stable without being degraded by enzymes and (ii) the ability to transform into multiple structures that have more functions than a single structure. Wang and his co-workers have presented an assembly-induced retention (AIR) effect [9] that the fibrous nanostructures exhibit longer blood circulation than the small molecules or their spherical counterparts. Thus, Wang's group designed a system where small molecules (or nanoparticles) transform to nanofibrils to achieve in situ self-assembly in vivo [2, 3, 9]. Yang's group designed a tandem molecular self-assembly to enhance the bioimaging in tumor area [12]. Xu's group have reported a hydrogel precursor which can be recognized by enzyme and then self-assemble into hydrogel

Table 3 Peptide self-assembly motifs

Interaction	Peptide category	Peptide name	Peptide sequence	Refs.
Hydrogen bond	Amyloid	Aβ(16-22)	NH_2-KLVFFAE-OH	[21]
		Aβ(30-40)	NH_2-AIIGLMVGGVV-OH	[59]
		β-turn(7-11)	NH_2-DSGYE-OH	[59]
		β-turn (23-27)	NH_2-DVGSN-OH	[59]
	Amphipathic	EAK16-II	Ac-AEAEAKAK-NH_2	[6]
		KFE8	Ac-FKFEFKFE-NH_2	[60]
		RADA16	Ac-RADARADA-NH_2	[61]
		Q11	Ac-QQKFQFQFEQQ-NH_2	[10]
		MAX1	VKVKVKVKV^{D}PPTKVKVKVKV-NH_2	[62]
$\pi - \pi$ interaction	Low molecular weight peptide	–	FF-OH	[5]
		–	FF-NH_2	[63]
			Boc-FF	[63]
			Fmoc-FF	[63]
Hydrophilic-hydrophobic interaction	Organopeptide hybrids	TGFBPA	HSNGLPLGGGSEEEAAAVVV (K)-CO$(CH2)_{10}CH_3$	[64]
		Filler PA	$H_3C(CH_2)_{14}$CO-VVVAAAEEE	[64]
		β-sheet PA	$CH_3(CH_2)_{14}$CONH-VVVAAAKKK-OH	[31]

in situ inside live cell [32]. The detailed mechanism will be accurately analyzed in the following sections.

36.3.2 In Situ Peptide Self-Assembly

Based on the functions of the nanomaterial, researchers often combine several different functional peptide motifs into one coherent system, shown in Fig. 1. In this section, we will introduce this strategy according to the different stimulating factors for peptide self-assembly.

36.3.2.1 pH Response

In order to meet fast metabolism and proliferation, the tumor cells require higher glycolysis than normal tissue. Accordingly, lactic acid has been overproduced which results into the low pH in tumor microenvironment. As displayed in Fig. 2, many peptide motifs have the characteristic of pH response. Michael Altman et al. have reported that EAK12-d (AEAEAEAEAKAK) and DAR16-IV (n-ADADADADARARARAR) have both stable α-helix and β-sheet conformations

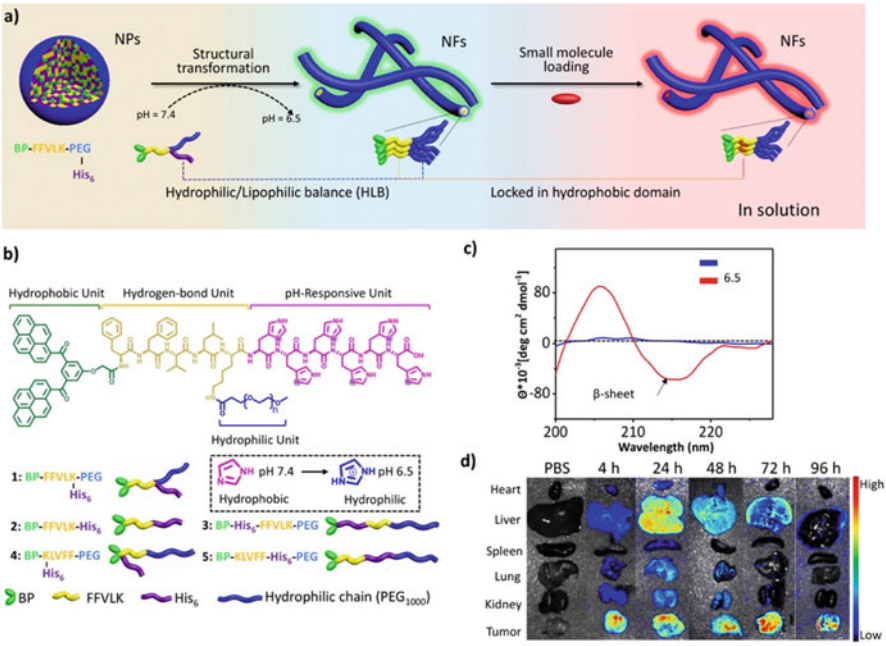

Fig. 2 (**a**) Schematic illustration of nanoparticle (NP) transformation at acidic pH. (**b**) The structures of designing molecules. (**c**) CD spectral characterization of transformation process with the β-sheet structure formation. (**d**) Time-dependent ex vivo fluorescence images of tumor tissues and major organs, such as the heart, liver, spleen, lung, and kidney, collected at 4, 24, 48, 72, and 96 h postadministration by PBS and NPs-1. (Reproduced with permission [3]. Copyright 2017, Wiley-VCH)

in different pH [23]. PloyHis has the different hydrophilic and hydrophobic ability in different pH. This feature has been used to design pH-responsive polymeric micelles by Zhang group to release paclitaxel in vivo [33].

Wang and his co-workers designed a pH-responsive peptide that led to the formation from nanoparticles (NPs) to nanofibers (NFs), from pH 7.4 to pH 6.5 (Fig. 2a) [3]. The peptide BP-FFVLK-PEG-His6 consists four motifs (Fig. 2b): (i) a bis-pyrene (BP) motif that is a hydrophobic unit and with green fluorescence emission by AIE effect [34], (ii) peptide sequence KLVFF which can aggregate to form β-sheet structure by hydrogen bond, (iii) a pH-responsive unit (His_6), and (iv) a hydrophilic chain (PEG1000). The His_6 is hydrophobic in pH 7.4 but is hydrophilic in pH 6.5. Therefore, this specific peptide sequence can form nanoparticles (NPs) through hydrophobic-hydrophilic interactions.

The change of pH in the microenvironment can trigger changes of hydrophobic-hydrophilic balance, which can lead to the transition from nanoparticles to nanofibers. The five peptides designed by the authors, shown in Fig. 2b, exhibit such morphology transformation. The NPs were injected into tumor-bearing mice by intravenous injection, and due to passive targeting mechanisms, the NPs transform

into NFs and construct nest-like hosts that covered the tumor region. These structures have β-sheet secondary structures (Fig. 2c). And the "nest" can restrain the tumor for more than 96 h (Fig. 2d). Moreover, small molecular drugs such as doxorubicin (DOX) can be loaded into the hydrophobic region of the β-sheet, thereby allowing the accumulation of small molecular drugs in the tumor region.

However, due to the complex environment in vivo, the pH is not stable and homogeneous. So the pH response strategy is not the most extensive, sensitive, and accurate method to design the peptide nanomaterials in vivo.

36.3.2.2 Enzyme Response

Due to the diversity of enzymes in the body and their predetermined expressions, a versatile strategy of stimulating peptide self-assembly is through enzymes. Take tumor, for example. Matrix metalloproteinases (MMPs), furin, and caspase family are overexpressed in many kinds of tumor area. Correspondingly, the peptide sequence shown in Table 2 can be cleaved by those enzymes. Recently, a concept, enzyme-instructed self-assembly (EISA), has been reported by Xu's group [35]. Then, this concept has been widely recognized by researchers and applied to design nanomaterials [36, 37].

Wang and his co-workers have reported a building block (Ppa-PLGVRG-Van 1), which can enhance the sensitive imaging of bacterial infection, to specifically target the bacteria-infected location in mice [38]. As the shown in Fig. 3b, the building block is composed of three moieties: (i) a signaling molecule pyropheophorbide-α, (ii) a peptide sequence Pro-Leu-Gly-Val-Arg-Gly (PLGVRG) which is a linker that can be cut by gelatinase, and (iii) the vancomycin (Van) as a targeting ligand. After intravenous injection in mice, the peptide compound targets and accumulates in the bacteria-infected location. The overexpression of gelatinase at the targeting location cleaves the peptide segment PLGVRG, then the signal molecule can aggregate into supramolecular structures through enhancement of hydrophobic and decrease in steric hindrance. These supramolecular aggregates can enhance photoacoustic signal at the infected location. This new discovery can provide new possibilities for biomedical applications.

Besides being directly responsive to enzymes, some peptides self-assemble after being stimulated by enzyme-responsive small molecules. Xu and his co-workers utilized ALP-triggered dephosphorylation to adjust the molecular hydrophobicity [32]. As shown in Fig. 4b, molecule 1 (NapFFKYp) is an amphipathic peptide with two moieties: the hydrophobic NapFF moiety and the hydrophilic KYp moiety (p is the abbreviation for phosphorous ester). Alkaline phosphatase (ALP) can recognize tyrosine phosphate residues and remove phosphorous ester by dephosphorylation. Molecule 2 NapFFK(NBD)Yp is a precursor. NBD is a fluorophore that can give more intense fluorescence in hydrophobic environment than in water. The precursor 2 can freely diffuse in solution and cannot self-assemble in the low concentration as shown in Fig. 4a. Then precursor 2 accumulates into cell, and dephosphorylation affords the corresponding hydrogelator 3. Hydrogelator 3 is more hydrophobic than the precursor, which endows hydrogelator 3 self-assembly to form nanofibers in the

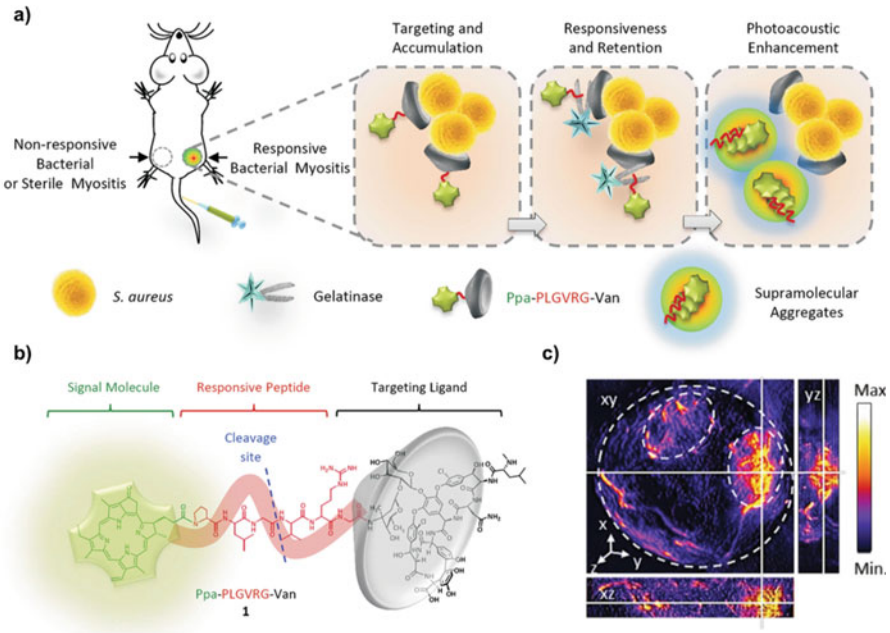

Fig. 3 (**a**) Illustration of bacterial infection imaging based on an in vivo aggregation strategy. First, the targeting molecule (Van) causes Ppa-PLGVRG-Van to accumulate at the site of responsive bacterial myositis; then, the gelatinase produced by gelatinase-positive bacteria in the infectious microenvironment cleaves the peptide linker, triggering self-aggregation in situ; finally, the supramolecular aggregates significantly enhance the photo-acoustic signal so that the bacterial infection can be detected by imaging. (**b**) Molecular structure of the designed peptide. (**c**) 3D reconstruction of an infected site (10^4cfu bacteria) 24 h after intravenous injection of 1 (200 μL, 2.0×10^{-4} M). (Reproduced with permission [38]. Copyright 2015, Wiley-VCH)

certain concentration. The fluorescent confocal microscope has shown the hydrogel in cells (Fig. 4).

Recently, cellular organelle self-assembled triggered cellular dysfunction has been proposed by Ryu and his co-workers [37]. The researchers took advantage of amphiphile peptide (Mito-FF) where pyrene is a fluorescent probe and Mito is a mitochondria-targeting hydrophilic moiety and positive charge carrier (Fig. 5b). As shown in the schematic illustration of Fig. 5a, after cellular diffusion in cytoplasm, the peptide does not self-assemble because the concentration is below the critical aggregation concentration (CAC). Then targeting and accumulating in mitochondria and self-assemble as a β-sheet structure because the peptide concentration in mitochondria is above CAC. The self-assembled nanofibers induced cellular dysfunction and triggered cell apoptosis. Compared with intracellular enzyme-instructed self-assembly (EISA), organelle localization-induced supramolecular self-assembly (OLISA) can increase local concentration and reduce the total dose of the materials to further reduce cytotoxicity. In order to further study the fibril formation inside the mitochondria, the researchers designed another peptide: Mito-FF-NBD, where

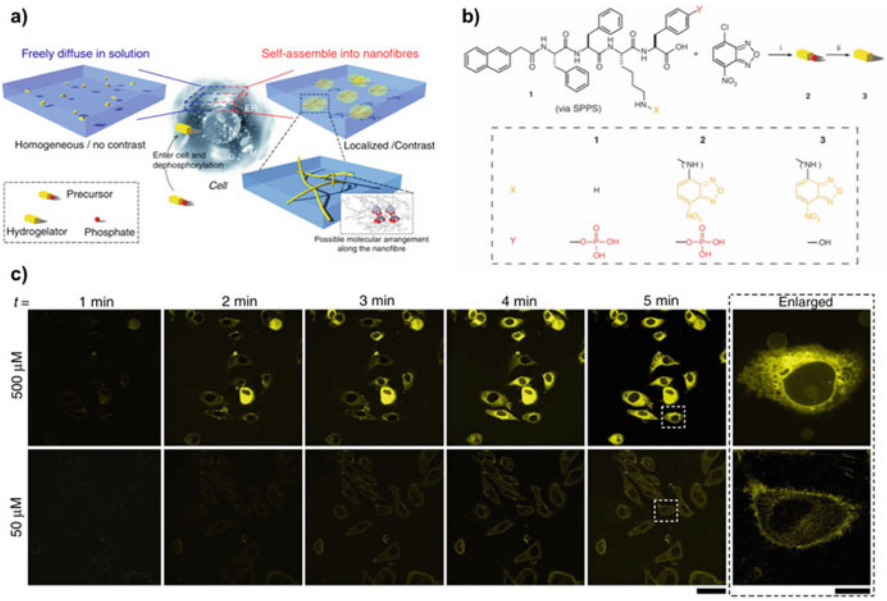

Fig. 4 (**a**) Principle of imaging enzyme-triggered supramolecular self-assembly inside cells. ER, endoplasmic reticulum; G, Golgi apparatus; L, lysosome; m, mitochondria; n, nucleus. (**b**) The synthesis route of the precursor **2** and the generation of the fluorescent hydrogelator **3** via an enzyme-catalyzed dephosphorylation. (i) Na_2CO_3, methanol, water, 50 °C, 2 h; (ii) ALP. (**c**) Enzyme-trigged self-assembly inside live cells. Fluorescent confocal microscope images show the time course of fluorescence emission inside the HeLa cells incubated with 500 or 50 μM of **2** in PBS buffer, which shows the different distribution of fluorophores inside living cells. Scale bar, 50 μm for time course panels and 10 μm for the enlarged panels. (Reproduced with permission [32]. Copyright 2012, Macmillan Publishers Limited)

4-nitro-2, 1, 3-benzoxadiazole (NBD) can release stronger fluorescence in hydrophobic conditions (Fig. 5d). The co-assembly of Mito-FF and Mito-FF-NBD can help to detect concentration-relative self-assembly (Fig. 5f is schematic illustration; Fig. 5 is confocal microscope of co-assembled system).

Compared with pH response strategy, EISA is more specific, accurate, and flexible. Enzyme response is the most important strategy to realize in vivo self-assembly.

36.3.2.3 Ligand-Receptor Interactions

Another in vivo self-assembled strategy is utilizing the ligand-receptor interaction. Some overexpressed ion or receptor in tissue microenvironment can influence the external supermolecule's spatial arrangement. For example, via the ligand-receptor interaction, the peptide Fmoc-GG_DA_DA can propagate by binding to vancomycin (Van) via hydrogen and further dissociation [39]. The Vans are released to solution, and binding peptide again and the polypeptide can autocatalyze themselves into aggregates (Fig. 6a, b). The resulting aggregates of Fmoc-GG_DA_DA can inhibit the

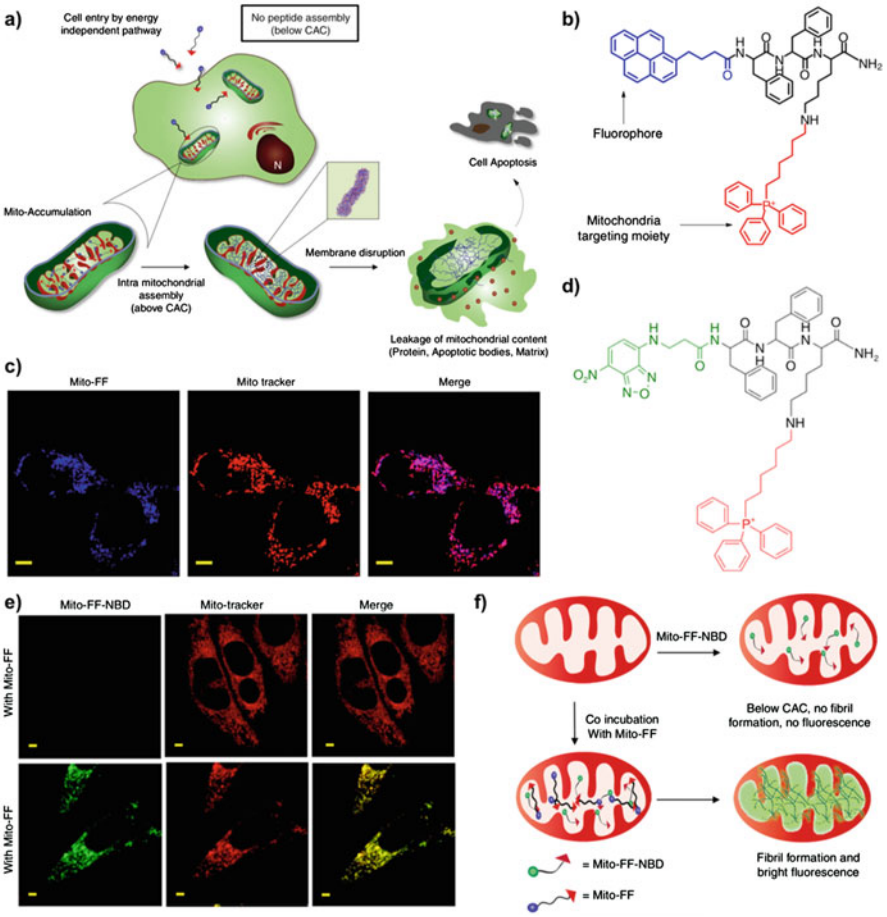

Fig. 5 (**a**) Intramitochondrial assembly of Mito-FF. The self-assembly process is driven by the increased mitochondrial membrane potential of cancer cells, leading to high mitochondrial accumulation of Mito-FF followed by self-assembly into fibrils. The intramitochondrial fibrils further disrupt the membrane, resulting in leakage of mitochondrial contents to the cytosol, which eventually induces cellular apoptosis. (**b**) Structural design of the mitochondria-targeting peptide amphiphile, Mito-FF, which is equipped with a pyrene group for fluorescence detection inside cells and TPP for targeting of mitochondria. (**c**) Mitochondrial co-localization of Mito-FF measured with MitoTracker Red FM shows high localization inside mitochondria (scale bar, 5 μm). (**d**) Molecular structure of Mito-FF-NBD. (**e**) Co-assembly inside mitochondria indicated by the bright green fluorescence of Mito-FF-NBD in the presence of Mito-FF (lower panel); however, such emission was not observed with Mito-FF-NBD alone (upper panel) (scale bar, 2 μm). (**f**) Schematic diagram showing co-assembly of Mito-FF-NBD with Mito-FF. (Reproduced with permission [37]. Copyright 2017, Macmillan Publishers)

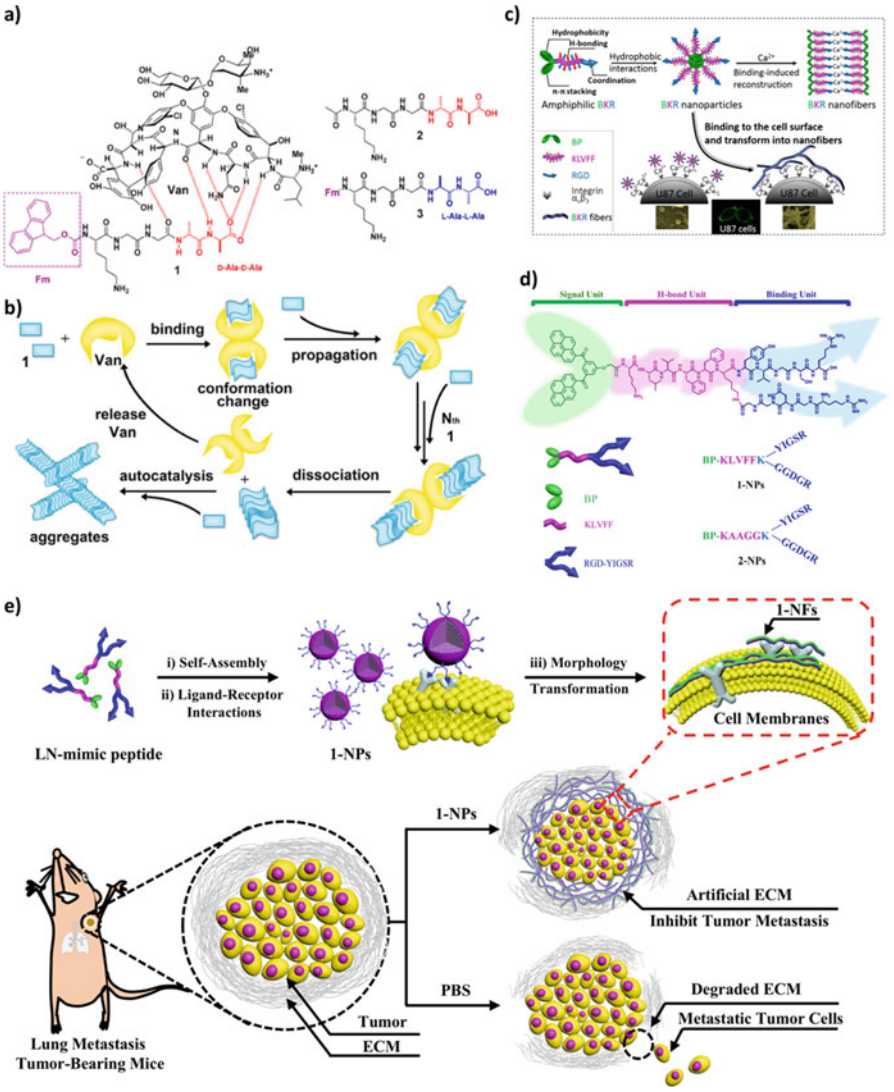

Fig. 6 (**a**) Structures of the ligand (Van), the receptor (a D-Ala-D-Ala derivative), and the relevant controls. (**b**) The ligand-receptor interaction-catalyzed molecular aggregation. (**c**) Schematic representation of metal ion binding-induced reconstruction of BKR nanoassemblies from nanoparticles to nanofibers in solution and on the cell surface. (**d**) Molecular structure and schematic illustration of peptide of AECM. (**e**) Schematic illustration of the biomimic construction of AECM based on transformable 1-NPs for highly efficient inhibition of tumor invasion and metastasis. (Reproduced with permission [2, 39, 40]. Copyright 2015,2017, American Chemical Society. Copyright 2016, Royal Society of Chemistry)

proliferation of HeLa cells with half maximal inhibitory concentration (IC_{50}) value of 184×10^{-6} m.

Recently, Wang and his co-workers utilized metal-ligand interaction to design a peptide BP-KLVFF-RGD (BKR) which can achieve a morphology transformation [40] (Fig. 6c). Due to the hydrophilic-hydrophobic interaction, the peptide BP-KLVFF-RGD (BKR) has a nanoparticle supermolecule arrangement. It is widely known that RGD can especially recognize integrin $\alpha_v\beta_3$, which is driven by the interactions between RGD and the metal ions (Ca^{2+}, Mg^{2+}). Because of the change of microenvironment, the BKR has a spatial arrangement transformation – from nanoparticles to nanofibers and then maintaining long retention on the cell surface.

To use this strategy, an artificial extracellular matrix (AECM) has been constructed to avoid tumor metastasis [2] (Fig. 6d, e). The authors designed a Y-type dual targeting motif (peptide sequence: RGD-YIGSR). Besides, the other two motifs are (i) signal molecule bis-pyrene (BP) and (ii) self-assembled KLVFF. BP and KLVFF are hydrophobic and RGD-YIGSR is hydrophilic. The amphipathic molecule has formed nanoparticle first. When the targeting unit binds the cancer cell surfaces, the ligand-receptor interactions triggered the molecular transformation from nanoparticles to nanofibers. The nanofibers have formed a net structure outside the cells which is similar to the ECM, so the authors call it the AECM (artificial ECM). The AECM cannot be degraded by enzymes, so it can inhibit the tumor invasion and metastasis.

Moreover, the ligand and receptor can also be a same object. Wang and his co-workers have intelligently designed a nanosweeper to degrade Aβ by upregulating autophagy. In this system, the ligand is the artificial synthetic KLVFF peptide, and the receptor is the KLVFF sequence of cerebral Aβ [22].

However, from now on, the mechanism of ligand-receptor interaction-induced transformation is not clear, which is the biggest barrier of the development in this field. Besides, the universality of this strategy needs further researches and discussions.

36.3.2.4 Redox Reaction

Besides pH, enzyme, and ligand-receptor interaction, other simulation can be used to contribute to the transformation of peptide in vivo. For example, Yang and his co-workers utilized redox reaction to achieve tandem molecular self-assembly [12]. The first self-assembly utilized alkaline phosphatase (ALP) to regulate molecule's hydrophilic-hydrophobic property, and the second self-assembly utilized GSH to cleave the disulfide bond (Fig. 7). This diphenylalanine (FF) system is reported frequently by Xu's group. In order to adjust the hydrophilic property of the molecule which concludes diphenylalanine (FF), they combined phosphorylated amino acids into the peptide chain to increase the hydrophilic property. The $-H_2PO_3$ can be cleaved by extracellular ALP. The change of hydrophilic will lead to the change of conformation. The second self-assembly of this work is a redox reaction. GSH is a widely known molecule existing in the tumor microenvironment. It can cut off the disulfide bond. RGD is a length of hydrophilic peptide. After cutting off sERGD, the self-assembled GFFY will self-assemble into nanofibers. This work could lead to the

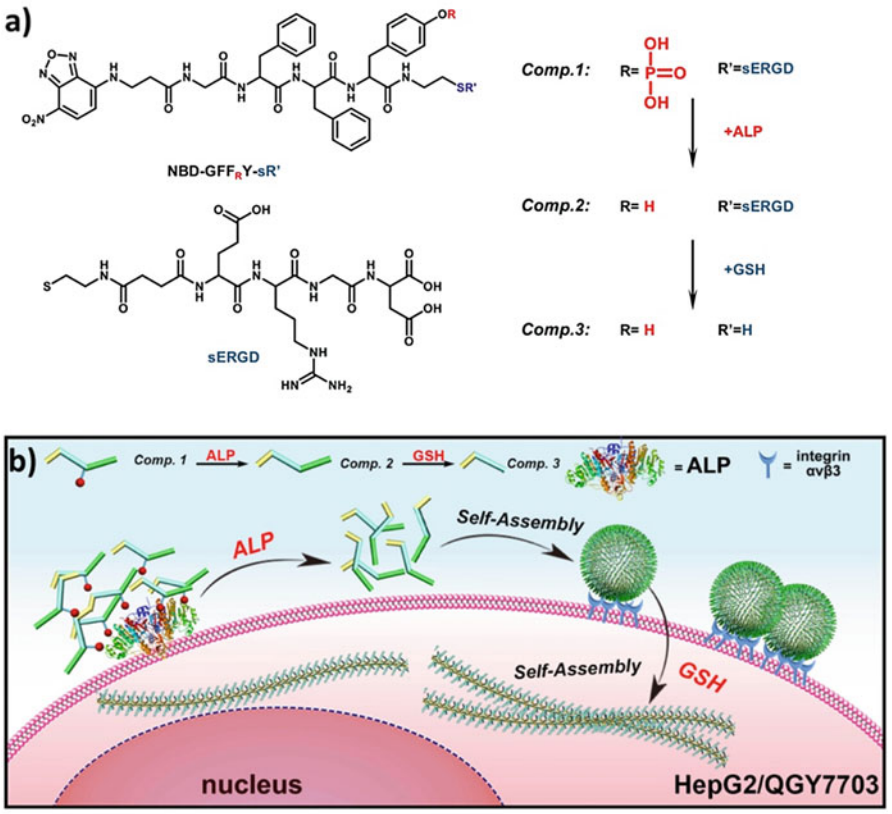

Fig. 7 (**a**) Chemical structures and schematic illustration of the conversion of 1 into 2 by phosphatase (ALP) and then of 2 into 3 by glutathione (GSH). (**b**) Proposed mode of the tandem molecular self-assembly in the extra- and intracellular environment of liver cancer cells. (Reproduced with permission [12]. Copyright 2017, Wiley-VCH)

development of supramolecular nanomaterials with improving performance in cancer diagnostics and therapy.

Redox reaction is a common method in small molecule drug chemistry field. Rao's group has reported a biorthogonal reaction to realize in situ self-assembly in vivo [41]. But it always utilizes small molecule motifs, not peptide. So we don't discuss it particularly in this chapter.

36.3.3 Polymer-Peptide Conjugates (PPCs) Self-Assembly In Vivo

Compared with polypeptide materials, polymer-peptide conjugate materials have more modified sites, better biocompatibility, and longer blood circulation time.

36.3.3.1 Thermosensitive PPCs

Thermosensitive polymers are widely used in biomedical material field. The thermosensitive polymers have lower critical solution temperatures (LCST). This material will be in a solid state if its environment temperature is below the LCST and a gel state when above. The LCST is also dependent on hydrophilic motifs; therefore, the more hydrophilic motifs attached to the polymer, the higher the polymer's LCST is. Based on this information, Wang et al. designed thermosensitive PPCs which can achieve intracellular sol-to-gel transition [42]. Their polymer-peptide conjugate is composed of three sections: a thermoresponsive polymer backbone (PNIPAAm), a hydrophilic peptide sequence whose goal is to modulate LCST, and a signal molecule side chain (Fig. 8). As shown in Fig. 8a, the LCST of the material is higher than 37 °C. So during blood circulation, the material does not aggregate into gels. When it penetrates into cells, the enzyme or other object will recognize the peptide response and cleave the hydrophilic motif, which can decrease the LCST. Then, the material aggregated into gels at 37 °C. This material can be used for in situ sensing and monitoring cellular physiological processes.

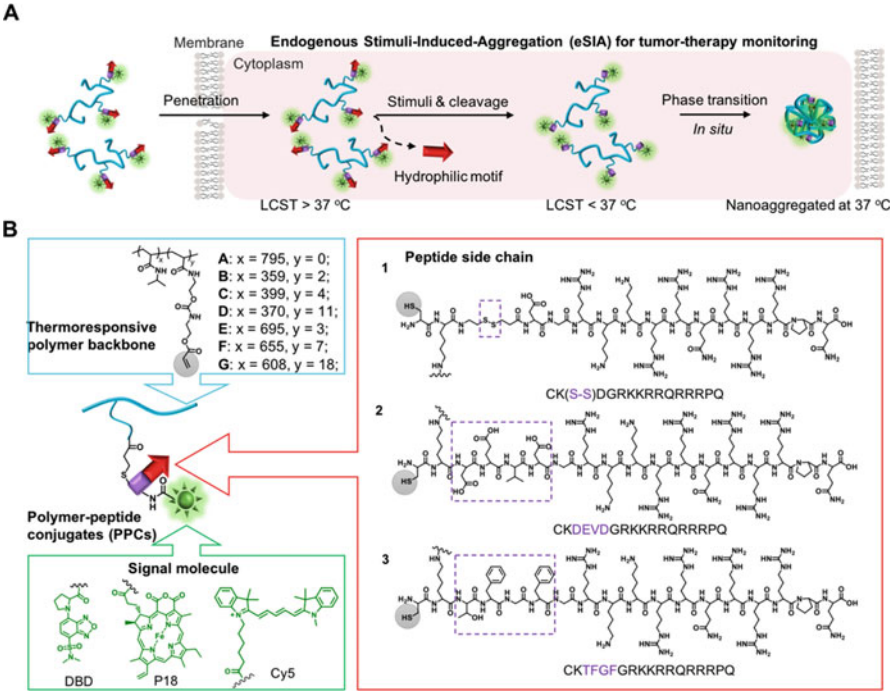

Fig. 8 (**a**) Schematic representation of stimuli-instructed construction of controllable nanoaggregates for monitoring tumor therapy response and (**b**) illustration of PPC structure and chemical structures of the thermoresponsive polymer backbone, peptide side chain, and signal molecule. (Reproduced with permission [42]. Copyright 2017, American Chemical Society)

ELPs, also a kind of thermoresponsive material, have been designed to combine with polymerization peptide and signal molecules by Wang group [24]. The end amino acid of the peptide is lysine, so monomer molecules have dissociative amide and amino. After entering the cytoplasm, the monomer can be catalyzed by TGase to conduct a polymerization process via the reaction between amide and amino. The newly formed amide makes the formation of peptide monomer to ELP topology-controlled nanostructures, including ELP random coil, ELP nanoparticle, and ELP gel, which can exhibit AIR effect and inspire scientist to explore new nanomaterials applied in living subject.

36.3.3.2 pH Response PPCs

The pH response PPC means a kind of nanomaterial that can delivery drugs as a vehicle and change structure when simulated by changing pH. The nanovehicles always have acid-sensitive bonds, for example, hydrazine, acetal, orthoester, and amide. Due to the better biocompatibility, scientists always used a polymer as a response motif. Wang and his co-workers have designed a pH-responsive polymer-peptide conjugate for monitoring the process of nanovehicles' intracellular acid-induced structural change [43]. The polymer-peptide conjugate was combined by (i) dextran (DEX) polymer, (ii) targeting peptide CGGRGD, and (iii) phenylboronic acid-modified P18 (NPBA-P18). The DEX is linked to the P18 by a phenylboronic linkage, a pH response bond that helps the nanovehicle transition at different pH levels.

Either thermosensitive or pH response PPCs, the main components are polymers. The peptide only plays one function role. Different types of materials can be linked together to form new nanomaterials, which can effectively enhance the versatility of materials.

36.4 Challenge and Outlook

In this chapter, we have introduced the principle of "in vivo self-assembly strategy," utilizing microenvironment response linker to control the deformation of materials and the release of drugs. Here, we focus on pH, enzyme, temperature, ligand-receptor interaction, and redox reaction response and introduce the details with specific articles as example. During the modulation of different peptide motifs, the peptide-based nanomaterials can (i) achieve different functions and (ii) self-assemble in different areas. Compared to peptide molecules, peptide self-assembled nanomaterials have high stability and long retention effect. These advantages afford the material's various applications: first, to be as a biosensor to monitor some intracellular activities; second, to be as a carrier to carry drugs into the desired location; and third, to be as a simulation agent to activate autophagy.

However, with the development of in situ construction peptide materials in vivo, some emerging challenges have appeared. (i) The mechanism of the self-assembled process in vivo is not clear, which is a barrier to achieve the precise control in vivo. And, the fuzzy mechanism will lead to difficulty in designing precursors. (ii) The

reason of disassembly and excretion of supramolecules in vivo is not available, which will increase the difficulty of toxicity evaluation. (iii) The interaction of peptide self-assembly with small molecules in vivo needs to be reported more so that the self-assembly can be applied in many theranostic fields.

In a word, "in vivo self-assembly" strategy will lead to benefits of designing nanomaterials which can be applied in theranostic regions and clinical medicine. To completely explore the treasures, a lot of efforts will be required by scientists.

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