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Confinement of Assemblies of Peptides by Chemical Reactions in Living Cells

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Abstract: The self-assembly of peptides plays an important role in optics, catalysis, medicine, and disease treatment. In recent years, peptide-based materials have exhibited great potential for cancer therapy and disease imaging due to their excellent biocompatibility, structural tenability, and ease of functionality. Peptides could self-assemble into diverse nanostructures in vivo triggered by endogenous stimuli, which initiated chemical reactions and self-assembled to achieve desired biological functions in the tumor microenvironment. This concept introduces the utilization of endogenous triggers to construct functional nanostructures in vivo and their corresponding biological applications. After briefly discussing the representative example of chemical reactions induced self-assembly of peptides in the living system, we describe the several stimuli triggered self-assembly for constructing therapeutic assemblies and serving as an imaging probe. Finally, we give a brief outlook to discuss the future direction of this exciting new field.

1. Introduction

Self-assembly is the spontaneous association process of molecules into highly ordered nanostructures driven by non-covalent interactions,[1] which is generally involved in the biological system, such as the construction of the cell membrane, DNA folding, and formation of higher-order structures of proteins.[2] Among the various building blocks to construct functional materials, peptides attract increased attention because of their low cost, convenient modification, and good biocompatibility. Peptides could assemble into highly ordered nanostructures driven by non-covalent interactions, including hydrogen bonding, aromatic-aromatic interactions, Van der Waals' interactions, and electrostatic interactions. External stimuli such as temperature, pH, light, enzymes, and other chemical triggers are usually involved in initiating the self-assembly of peptide,[3] thus controlling chemophysical properties of assemblies.

In the last two decades, the development of peptide self-assembly has promoted their applications in many areas, including optics, catalysis, biology, clinical medicine,[4] and disease treatment.[5] Differing from the controlling self-assembly of peptide in vitro, using endogenous triggers to initiate the self-assembly of peptides in situ of living systems attracted more attention in recent years. Cancer is a kind of life-threatening disease, and the development of novel strategies for cancer therapy has been extensively studied in the past decades.[6] Cancer cells possess many unique characteristics different from normal cells, including overexpressed enzymes, a unique pathological microenvironment, and the production of reactive oxygen species (ROS).[7] Developing peptide self-assembly in tumor cells could take advantage of the unique physiological conditions of the tumor microenvironment, thus achieving superior therapeutic efficacy towards cancer cells. Enzyme-instructed self-assembly (EISA) of peptide is the first example introduced into living cancer cells, which offers a new strategy to develop assemblies of peptide in situ to control the cell fate.[8] Based on the strategy, many types of enzymes and chemical agents (alkaline phosphatase (ALP),[9] matrix metalloproteinase-7 (MMP-7)[10], H_2O_2, GSH) overexpressed by different cancers have been used to induce peptide self-assembly in living cells.[11] Moreover, the EISA based self-sorting strategy has also been developed in living cells, then achieved the tumor penetrating and organelle targeting property.[12] Though extensive efforts have been focusing on endogenous stimuli-induced self-assembly in living cells after pioneering work reported by Xu and co-workers[8], the design of the cellular compartment-specific self-assembly system is in its infancy.

In this concept, we first focus on the single stimuli (enzyme, pH) responsive peptide self-assembly, then we give a brief introduction of peptide self-assembly in living cells triggered by two stimuli (enzyme-pH, enzyme-enzyme, enzyme-ROS/GSH), and finally, we will give a summary and put forward our perspective. We show our sincere apology in advance for some works are inadvertently absent from this work and hope the reader will inform us their works so we can cover the related content in the future work.

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2. Chemical Reactions Induced Self-Assembly of Peptides in the Living System

2.1 Enzyme-induced self-assembly of peptide in cells

Based on the EISA strategy, Xu and co-workers[9] introduced a subcellular targeting motif into the peptide for organelle-specific accumulation of assemblies, which could be used to kill cancer cells at a lower concentration without drug resistance. The peptide (NBD-FFpYK-TPP, **1P**) consists of a self-assembly sequence (FFYK), the ALP responsive tyrosine phosphate (pY), an environment-sensitive fluorophore (NBD, 4-nitro-2, 1, 3-benzoxadiazole), and a mitochondria targeting motif (TPP) (Figure 1A). After uptake into the cancer cells, **L-1P** (or **D-1P**) could convert into **L-1** (**D-1**) catalyzed by overexpressed ALP, which further self-assembles into nanofibers. CLSM images reveals that the formed assemblies could escape from lysosomes and accumulate at mitochondria, resulting in cancer cell death selectively (Figure 1B). Furthermore, western blot analysis demonstrates that mitochondria dysfunction could release cytochrome c into cytoplasm, resulting in the activation of caspase cascade reaction, then induce cell death. Significantly, the strategy could minimize the resistance of cancer cells. The work offers a novel approach to integrate enzymatic self-assembly and mitochondria targeting, improving the efficacy of cancer therapy.

The EISA strategy could also target the downregulation of cancer cells.[13] The same group designed an EISA precursor **2-OMe-OP**, which contains a carboxylesterase (CESs) and an ALP responsive motif. Under the action of ALP and CES, the **2-OMe-OP** could transform into **2-OMe-OH** and **2-OH-OP** sequentially. Moreover, **2-OMe-OH** could self-assemble into nanofibers, while the cleavage of the ester bond by CES induces the disassembly of nanofibers (Figure 1C). The measurement of critical micelle concentration (CMCs) reveals that the self-assembly ability of **2-OMe-OH** is higher than **2-OMe-OP**, **2-OH-OH**, and **2-OH-OP**, respectively, which is related to their cytotoxicity against cancer cells. After incubating **2-OMe-OP** with HepG2 (human hepatoellular carcinomas) cells upregulating the CES, the IC_{50} of **2-OMe-OP** is 15 times higher than the OVSAHO (human ovarian carcinoma) cells, which downregulates the CES, indicating **2-OMe-OP** could selectively target the downregulation of CES. This work offers the first example of a rational design peptide to target the downregulation of cancer cells.

Confinement of self-assembly of peptides into specific organelle of lysosome has yet to be reported until recently. Wang and co-workers[14] achieved the spatiotemporal control of the peptide self-assembly inside the lysosome of living cells. They designed peptide **3**, which contains three parts, a self-assembly region (Nap-wyf), the cathepsin B (CTSB) responsive sequence (GFRARGK), and C-terminal glycosylation (mannose), and the mannose group could increase the stability of **3** in the serum and inside cells (Figure 2A). After screening the peptide sequence, they find that the stability and glycosylation of peptides in serum correspond to their cytotoxicity against human astroblastoma cells-U87MG cells. After uptake into the cells through endocytosis, peptide **3** can be cleaved by CTSB, and the resulting part self-assembles to form nanofibers inside lysosomes. The authors also find that the stability of peptides could influence the accumulation of assemblies in the serum. The formation of assemblies at the specific site could further induce the lysosomal membrane permeabilization (LMP), then activate the downstream cell death pathway. This work paves the way for designing molecular self-assembly inside the lysosome and could be a strategy for solving diseases related to the lysosome.

In another contribution, Du and co-workers[15] developed tyrosinase-induced intracellular self-assembly of tripeptide (Figure 2B), which could disturb the self-polymerization process of tubulin and induce the apoptosis of drug-resistant melanoma cancer cells. Tripeptide FFY (**4**) could be oxidized by tyrosinase to form FFY dimer (**m4**) in vitro, and the self-assembly processes proceed inside cancer cells which overexpress tyrosinase could result in the formation of toxic nanostructures, then inducing the melanoma cancer cell death selectively. CLSM results

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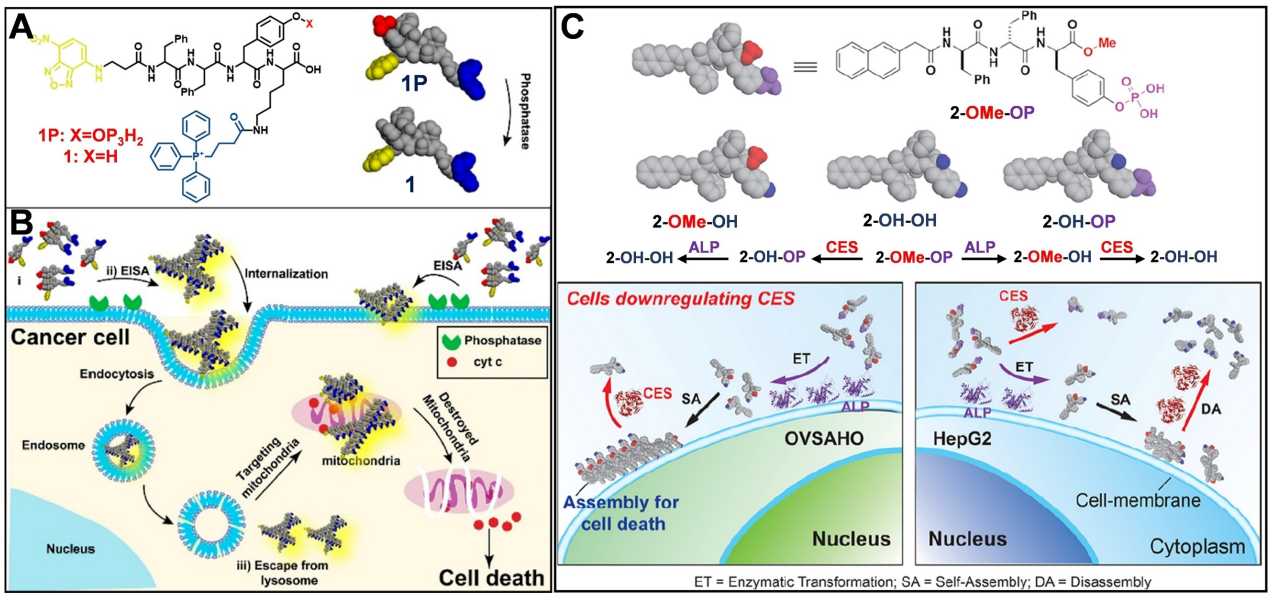

Figure 1. A) Molecular structure of **1P** and **1**; B) Schematic illustration of the integration of EISA and mitochondria targeting. Reproduced with permission from Ref.[9] Copyright 2016, American Chemical Society. C) Molecular structures of precursor **2-OMe-OP**, the hydrolysis products (**2-OH-OH** and **2-OH-OP**), and the schematic illustration of targeting downregulation of cells for cancer therapy. Reproduced with permission from Ref.[13] Copyright 2017, American Chemical Society.

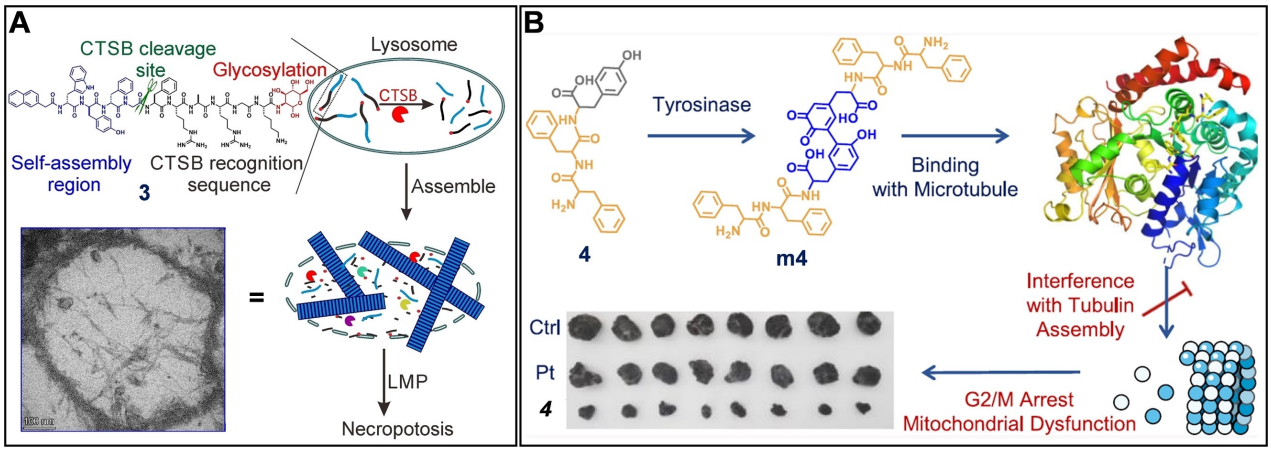

Figure 2. A) Schematic illustration of CTSB responsive self-assembly peptide **3** enters into the lysosomes via endocytosis, then forming the assemblies inside the lysosome and inducing LMP and necrocytosis of cancer cells. Reproduced with permission from Ref. [14]. Copyright 2021 Wiley-VCH GmbH. B) Tripeptide **4** is oxidized into **m4** catalyzed by tyrosinase, then **m4** binds with intracellular microtubule, disrupting the tubulin assembly and inducing the severe G2/M arrest and mitochondrial dysfunction, which could achieve the efficient therapy of drug-resistant melanoma tumors. Reproduced with permission from Ref. [15]. Copyright 2022 American Chemical Society.

reveals that the **m4** could colocalize with the intracellular microtubule, and the formation of **m4** could also inhibit the microtubules' formation. Furthermore, the cell division cycles experiment in mouse melanoma cells-B16F10/Pt cells reveals that the G2/M arrest is observed after treatment with **4** (13.9% higher than the control group). They also use JC-10 probe to investigate the intracellular mitochondrial membrane potential. Compared with the control group and cisplatin group, the ratio of the fluorescence intensity between red and green shows a significant decrease for the cells treated with **4**, indicating the dysfunction of mitochondria. The in vivo anticancer experiments indicate that assemblies of **4** could prevent tumor growth after intravenous injection. The tyrosinase-induced tripeptide self-assembly exhibits efficient apoptosis towards drug-resistant melanoma, offering a new strategy to treat drug-resistant cancer cells.

2.2 Acidic microenvironment triggered self-assembly

The acidic condition in the tumor microenvironment could be used to trigger the cleavage of the chemical bonds and then

induce the self-assembly of peptides. Liang and co-workers[16] conjugated the polymers with self-assembled peptides through an acid-responsive acetal bond (**5**). After loading the antigenic peptide (AP), the proton-driven morphology transformation occurs at an acidic endosomal environment and induces the endosomal membrane disruption, which could deliver the AP into the cytoplasm and induce the strong immune response of the tumor vaccine (Figure 3A, B). The polymer-peptide conjugate **5** exhibits the pH-dependent morphology transformation behavior. The nanospheres formed by **5** could transform into rigid nanosheets with a length or width of several micrometers by changing the pH from 7.4 to 5.6. The authors also demonstrate that **5** could co-assemble with AP, as evidenced by the enhancement of cytosolic peptide fluorescence from CLSM experiments. The co-assemblies of **5** and AP could deliver AP into the cytosol and promote the proliferation of DCs, inducing the prolonged surface antigen presentation and cross-presentation to $CD8^+$ T-cells. In vivo experiments indicate that the co-assemblies could facilitate AP delivery to draining lymph nodes through the NLRP3-inflammasome pathway. Moreover, the proton-driven nanotransformer-based vaccine can inhibit the tumor growth of both B16F10-OVA melanoma-bearing mice and human papilloma virus-E6/7 (TC-1) tumor-bearing mice. After combing the vaccine with anti-PD-L1, the growth of B16F10 tumor can be inhibited significantly, and the mice survive for longer than 83 days. The proton-driven nanotransformer-based vaccine provides an efficient and safe strategy for cancer immunotherapy.

Biomolecular condensate plays an important role in the biological system, which has yet to be achieved in materials science. Wang and coworkers[17] developed a general approach to construct biomolecular condensate (hydrogels) in lysosomes of living cells, which could be used for cancer therapy and address drug resistance. They design the lysosome targeting molecule **6**, which contains three parts, the segment derived from insulin protein could form a β-sheet structure at acidic pH, Nap offers aromatic-aromatic interactions during the self-assembly process, and the C-terminal glycosylation could increase the stability of peptide (Figure 3C). In vitro experiments indicate that the CMC of **D-6** decreases significantly when the pH reduces from 7.4 to 5.0. Furthermore, the formed nanostructures changes from nanospheres to nanofibers (Figure 3D). When the **6** is uptake by cancer cells through caveolae-dependent endocytosis, the phase transition from solution to hydrogels could be observed at lysosomes, increasing the permeability of lysosomes (Figure 3E). The authors also demonstrate that the acidic environment-induced self-assembly of peptides at lysosome could enhance the efficiency of chemotherapy drugs against drug-resistant cells, indicating the potential application for addressing multidrug resistance in cancer therapy.

2.3 Combination of the acidic microenvironment and other stimuli

Besides a single trigger, combining the acidic microenvironment and other stimuli in spatiotemporal control remains challenging. Weil and coworkers[18] reported the formation of fibrillar architectures through multistage self-assembly of the peptide. The designed peptide **7** contains a trans-activator of transcription (TAT) sequence and a pro-assembling sequence, these two parts are complexed by the pH-dependent boronic acid-salicylhydroxamate (Figure 4B). After entering into cells via endocytosis, the acidification could induce the release of the pro-assembling peptide from the complex. The elevated endogenous H_2O_2 induces the self-assembly of the peptide sequentially because of the sensitivity the boronic acid in the presence of H_2O_2. Finally, the fibrillar architectures are formed inside cells through multi-stage self-assembly (Figure 4A). They

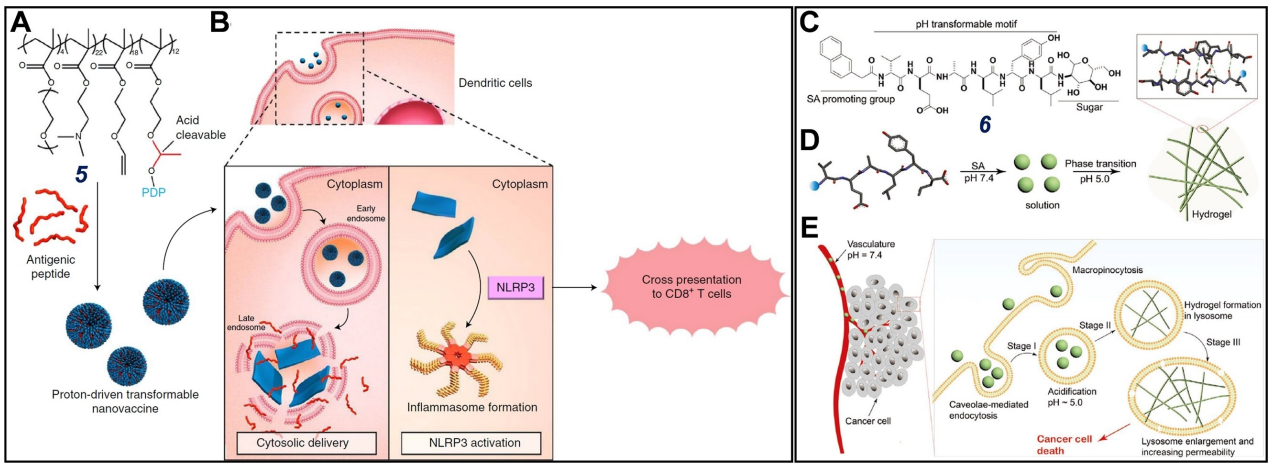

Figure 3. A) The composition of proton-driven nano transformer-based vaccine. B) The acidic endosomal environment triggers the cleavage of peptide, then re-assembles into nanosheets. Morphology transformation induces the disruption of the endosomal membrane and delivers the AP into the cytosol, achieving the NLRP3 pathway and promoting antigen processing. Reproduced with permission from Ref. [16]. Copyright 2020 Nature Publishing Group. C) Molecular structure of **6**. D) pH-responsive self-assembly property of **6**. E) Schematic illustration of peptide condensates' construction in lysosomes of cancer cells. Reproduced with permission from Ref. [17]. Copyright 2021 Wiley-VCH GmbH.

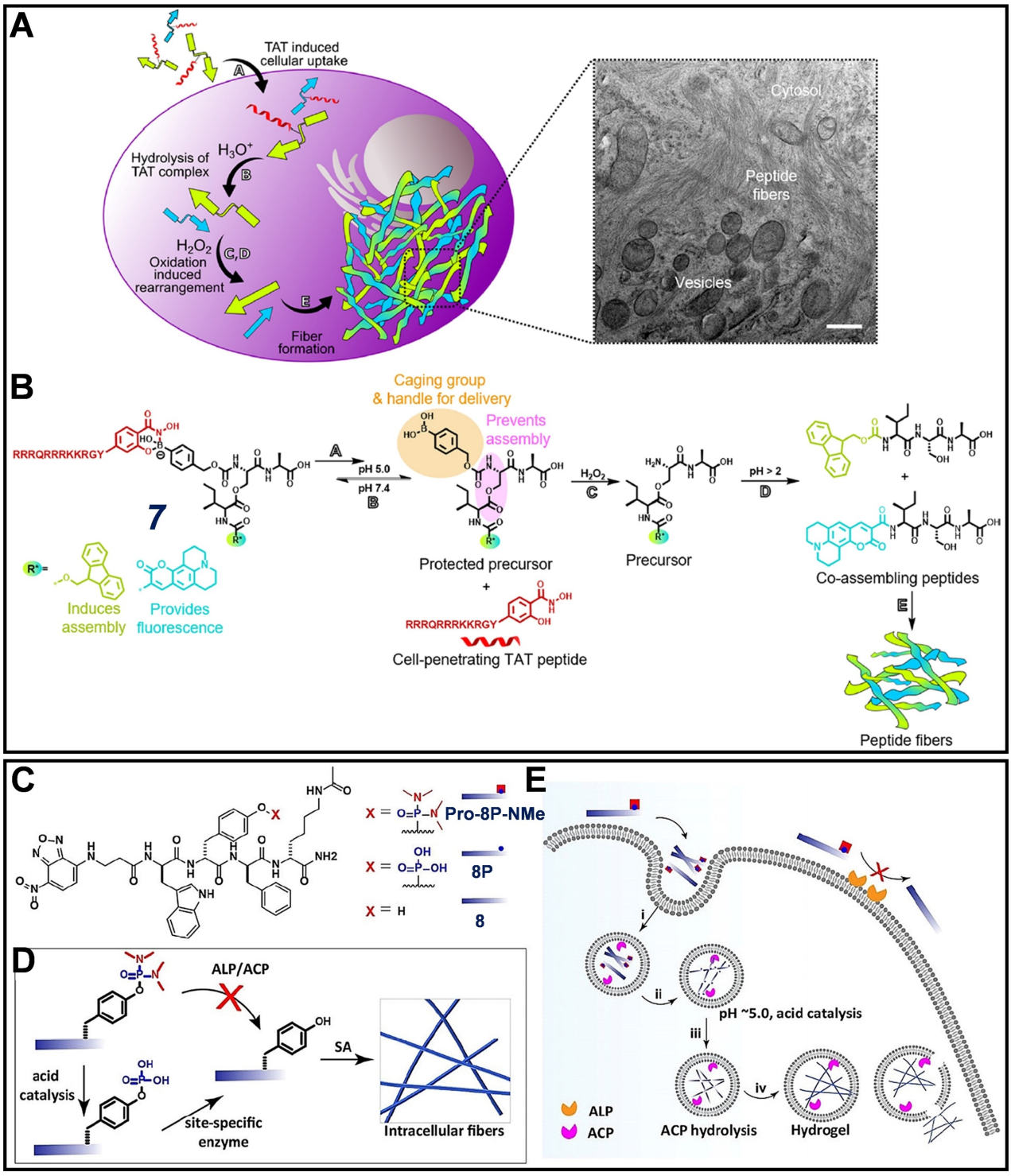

Figure 4. A) Schematic illustration of the Intracellular co-assembly of peptide **7** inside cells. B) Chemical reactions and self-assembly process of **7**. Reproduced with permission from Ref. [18]. Copyright 2020 American Chemical Society. C) Molecular structure of acid catalytic and enzyme-induced self-assembly peptide **Pro-8P-NMe**. Illustration of multistage self-assembly D) in vitro and E) inside living cells. Reproduced with permission from Ref. [19]. Copyright 2021 Wiley-VCH GmbH.

also altered peptide **7** with the different terminal groups to obtain a sufficient fluorescence signal inside cells, Fmoc promoted the self-assembly of peptides, while the Coumarin 343 (C343) provided fluorescence of self-assembled nanostructures. The co-assemblies of Fmoc and C343 modified **7** exhibit both pH and H_2O_2-responsive properties, as demonstrated by HPLC spectra and TEM images. After incubating **Fmoc-7**/**C343-7** with A549 cells (human lung carcinoma cells)

for 2 h, the Annexin-V appears at the cell membrane, demonstrating the cells underwent apoptosis. After 4 h, the fluorescence of propidium iodide is observed in the nuclear region, indicating the disruption of the cell membrane, which is related to late-stage apoptosis. Bio-TEM images also reveals a nanofibrous network inside the cells, contributing to the programmed cell death. The multistage self-assembly platform is expected to achieve multifunction in cancer therapy and drug delivery.

To precisely control the formation of molecular assemblies in living cells for developing functional higher-order structures remains challenging. Taking advantage of acid-catalyzed hydrolysis of phosphodiamidate derivatives, Wang and coworkers[19] designed precursors that resist the hydrolysis by extracellular and cytoplasmic enzymes. In the acidic environment of living cells, the hydrolysis of the P–N bond of precursors releases the native substrate of the enzyme, thus enabling the enzyme to induce the nanofibers' formation inside the living cell where the enzyme exists. The designed peptide **Pro-8P-NMe** (Figure 4C) shows the acid-catalyzed hydrolysis and enzyme-induced self-assembly property (Figure 4D), as evidenced by LC–MS spectra and time-dependent TEM experiments. When the **Pro-8P-NMe** enters into the lysosomes through micropinocytosis-mediated endocytosis, the acidic environment could cleave the NMe group, releasing the substrate of enzyme **8P**. Furthermore, the acid phosphatase (ACP) inside lysosomes could further induce the enzymatic reaction from **8P** to **8**, forming the nanofibers inside the lysosomes (Figure 4E). Bio-TEM experiments reveals the formation of nanofibers at the surrounding of lysosomes. This work serves as a general and efficient platform to precisely control the formation of assemblies in living cells.

2.4 Combination of enzyme and other stimuli

Liang and co-workers[20] used extracellular ALP and intracellular glutathione (GSH) to build different nanofibers in the cell environment, which provides a platform for regulating nanostructures using two kinds of stimulus that overproduced by cancer cells. Yang and co-workers[21] used ALP and GSH as two tandem stimuli to modulate the self-assembly property of peptides in liver cancer cells. The peptide **9** (NBD-GFFpY-ss-ERGD, Figure 5A) responds to dual stimuli and converts into **comp. 10** (NBD-GFFY-ss-ERGD) after addition of ALP, then self-assembles into uniform nanoparticles. **Comp. 10** could be further catalyzed by GSH and transformed into the **comp. 11** (NBD-GFFY-thiol), forming hydrogels consisting of nanofibers (Figure 5B). The authors chose HepG2 cells as a model system since this cell line overexpresses extracellular ALP and intercellular GSH. CLSM experiment reveals the formation of nanoparticles around cell surface after incubating **comp. 9** with HepG2 cells for 0.5 h. The formed nanoparticles further transform into nanofibers, which could be observed near the nuclear membranes after 4 h incubation. The results demonstrate the occurrence of tandem self-assembly in liver cancer cells. The cell experiment further revealed that the tandem self-assembly could lead to liver cancer cell death selectively. This work offered a new way to use a tandem strategy for cancer therapy.

In another contribution, Liang and co-workers[22] developed an ALP and CES dual-responsive prodrug, which could enhance the efficacy of mild-temperature photothermal therapy (PTT) by inhibiting autophagy. The designed prodrug **12P-HCQ** contains three parts: the ALP responsive self-assembly sequence (Phe-Phe-Lys-Tyr(H_2PO_3)-OH), a NIR dye (cypate) that serves as a photothermal fluorophore, and an autophagy inhibitor hydroxychloroquine (HCQ). **12P-HCQ** could convert into **12-HCQ** and **12** sequentially in the presence of ALP and CES, then the TEM images reveal the formation of nanoparticles after successive

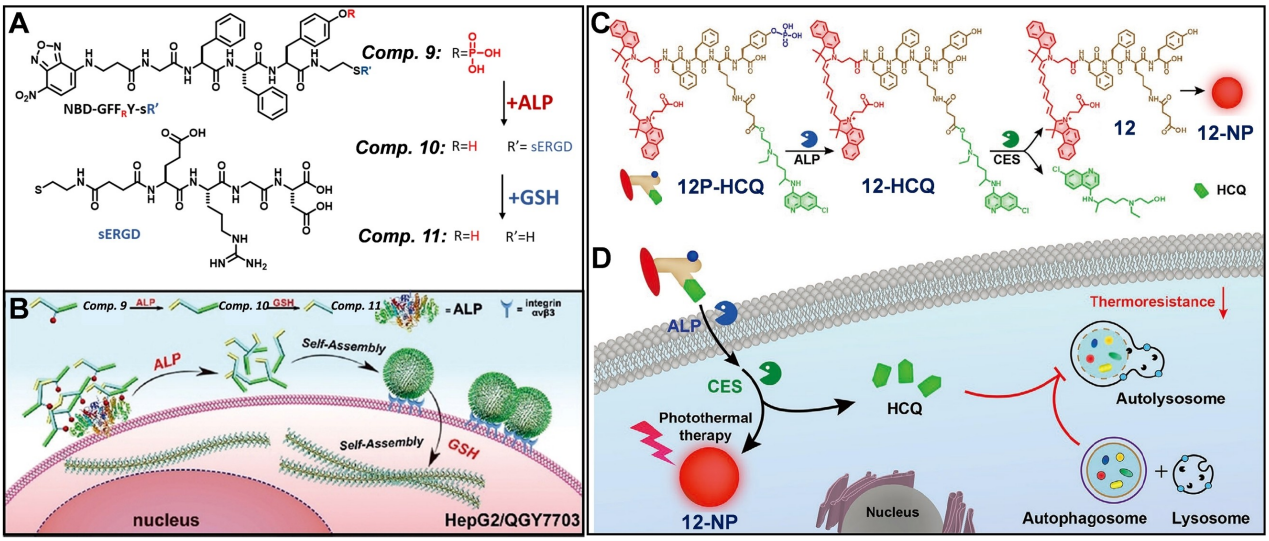

Figure 5. A) Molecular structures of precursor **9**, **10**, and **11**; B) Illustration of the tandem molecular self-assembly of **9** in liver cancer cells. Reproduced with permission from Ref. [21] Copyright 2017 Wiley-VCH GmbH. C) Chemical structures of **12P-HCQ** and its enzymatic transformation. D) Illustration of the working mechanism of **12P-HCQ** in autophagy-inhibited mild-temperature PTT. Reproduced with permission from Ref. [22]. Copyright 2021 Wiley-VCH GmbH.

chemical reactions, and the HCQ could be released during the self-assembly process (Figure 5C). After uptake by HepG2 cells that overexpressed ALP and CES, the ALP at the cell outer membrane and CES in the cytoplasm could promote the formation of **12-NP**, then the localization and accumulation of cypate exhibit an improved photothermal effect under NIR light irradiation (Figure 5D). Meantime, the release of HCQ could inhibit the autophagy process, decreasing the thermoresistance of cells. The in vivo photothermal tumor imaging reveals that the thermal signals could be observed obviously in the tumor areas after 8 h post-injection, indicating the **12P-HCQ** could target tumors efficiently. The tandem enzymatic self-assembly and autophagy inhibition strategy offers a novel way to design smart prodrugs for cancer therapy.

Wang and co-workers[23] designed two kinds of peptide-cyanine conjugates (**P-13Cy** and **P-14Cy**) (Figure 6) with controlled cyanine aggregation. The designed molecule consists of an X-linked inhibitor of apoptosis protein (XIAP) recognition sequence (AVPIAQK), a caspase-3/7 cleavable linker (DEVD), a self-assembly sequence (KLVFFAECK for **P-13Cy**, GCKLVFFAECG for **P-14 Cy**), and the cyanine substitution (1 for **P-13Cy**, 2 for **P-14Cy**). After targeting the XIAP and activating caspase-3/7 inside cells, the stacking pattern and aggregation state of **Pr-13Cy** and **Pr-14Cy** could be regulated. The results indicate that the **Pr-13Cy** can not form the aggregate with a defined structure, while the **Pr-14Cy** achieves H-aggregates. Based on the different self-assembly properties of two peptide-cyanine conjugates, **Pr-13Cy** can emit fluorescence, and the relative fluorescent quantum yield could reach up to 9.5 %, which can be used in tumor imaging. Meanwhile, the **Pr-14Cy** shows enhanced vibrational relaxation and photothermal effects, and the photothermal conversion efficiency is 3.4 times of **Pr-13Cy** self-assemblies. The in vivo self-assembly of the peptide could be used to precisely regulate the aggregation state of peptides and apply them in different areas.

Zhao and Wang[24] groups further designed a self-assembling selenopeptide, which combines chemotherapy and immunotherapy, achieving the enhanced tumor inhibition efficiency of peptides. The selenopeptide Sec(Dod)$_2$KGPLGVRGRGD (**15**) contains three parts (Figure 7A): a tumor-targeting group (RGD), a matrix metalloproteinase-2 (MMP-2) responsive cleavable linker (PLGVR), and a ROS

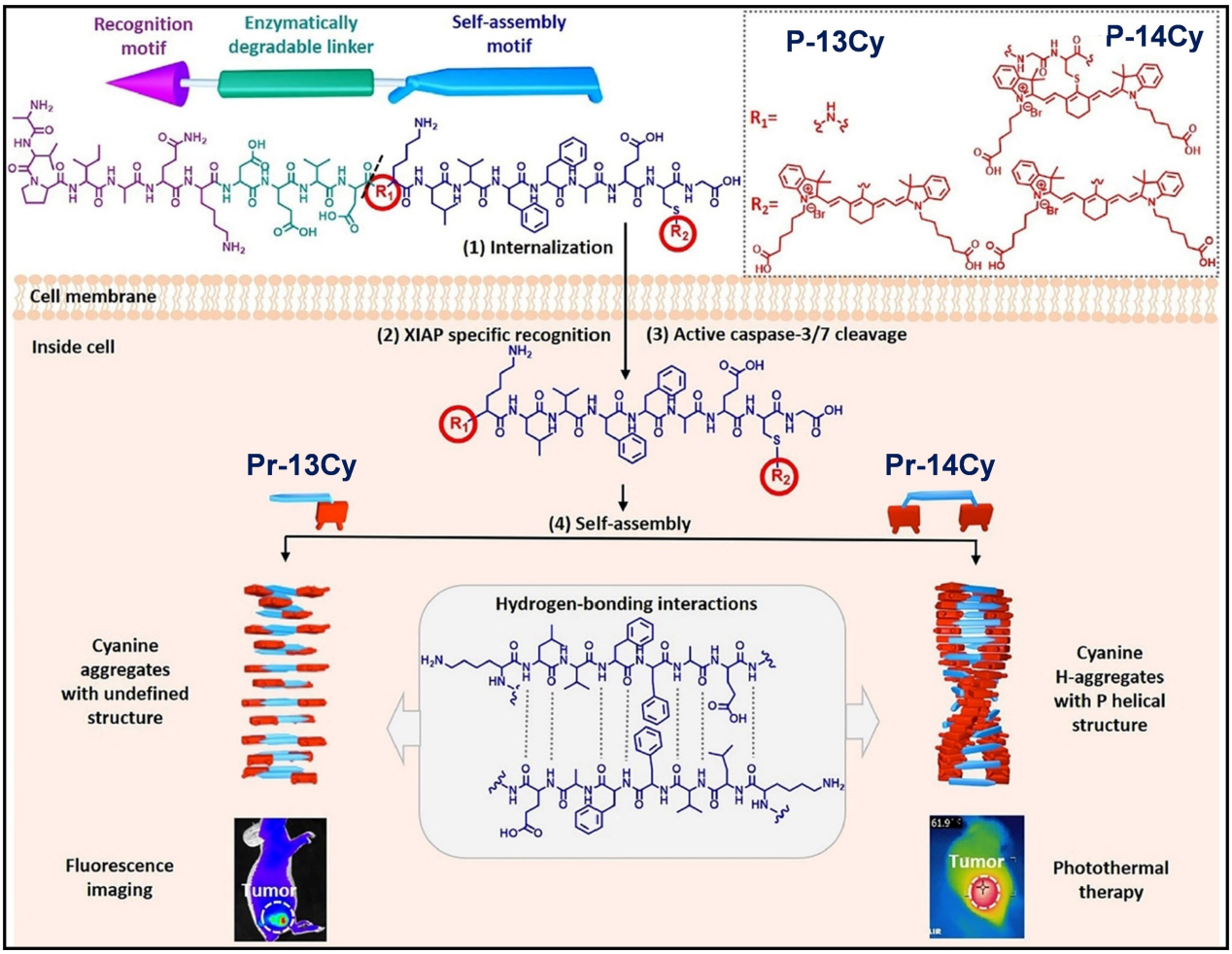

Figure 6. Molecular structures of **P-13Cy** and **P-14Cy**, and the XIAP and caspase-3/7 induced self-assembly process inside cells. The formed **Pr-13Cy** and **Pr-14Cy** can be used in fluorescence imaging and photothermal therapy of tumors, respectively. Reproduced with permission from Ref. [23]. Copyright 2021 Wiley-VCH GmbH.

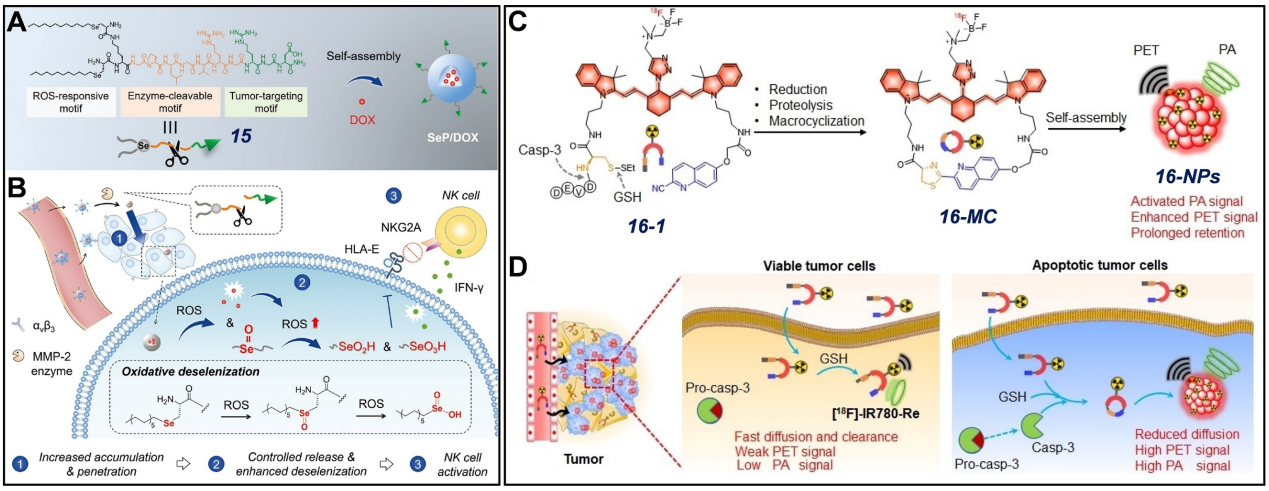

Figure 7. A) Molecular structure of selenopeptide **15** and the formation of nanoparticles with DOX. B) The MMP-2 enzyme and ROS responsive self-assembly and release of DOX inside tumor cells. Reproduced with permission from Ref. [24]. Copyright 2022 Wiley-VCH GmbH. C) Chemical structure of the designed PAI/PET bimodal probe **16-1**, and the self-assembly processes. D) Schematic illustration of the mechanism of **16-1** used for PET and PA bimodal imaging of tumor apoptosis in vivo. Reproduced with permission from Ref. [26]. Copyright 2022 Wiley-VCH GmbH.

responsive alkyl chain modified selenoamino acid tail ($Sec(Dod)_2K$). After capturing doxorubicin (DOX), the selenopeptide **15** could form SeP/DOX nanoparticles. The formed nanoparticles recognize the overexpressed $\alpha_v\beta_3$ integrins of tumors, accelerating the tumoral accumulation after systemic administration. After hydrolysis by the overexpressed MMP-2 of cancer cells, the DOX can be released quickly in tumor cells. Meanwhile, the ROS in the tumor microenvironment could decompose the selenopeptide, leading to the oxidative metabolites, including alkyl seleninic acids, which result in the activation of the immune response of natural killer (NK) cells (Figure 7B). The CLSM results of MDA-MB-231 cell (human breast cancer cells) spheroids indicate that the MMP-2 enzyme enhances the tumor penetration of SeP/DOX nanoparticles, and the In vivo experiment demonstrate that the tumor inhibition ratio could reach 86%, which is much higher than the free DOX and the combination of DOX and thiopeptide/$(Dod)_2$KGPLGVRGRGD group, indicating a synergistic effect between the DOX and selenopeptide. This work provides a new type of nanoparticles based on the self-assembly of selenopeptide, offering new building blocks for supramolecular assemblies of small molecules.

Ye and co-workers[25] reported the tumor-targeting and caspase-3 activatable photoacoustic imaging (PAI) probe using the 1-cyano-6-hydroxyquinoline (CHQ)-Cys macrocyclization reaction. Using such a probe, they obtain a high-resolution 3D reconstruction image, which can be used to investigate the activity of caspase-3 and the distribution of caspase-3 inside apoptotic tumors. Based on the above discovery, they recently develop a new stimuli-response photoacoustic (PAI)/PET bimodal imaging probe.[26] The designed PAI/PET probe (**16-1**) consists of a triazole-IR780 scaffold modified with four functional components (Figure 7C), including a CHQ group, a D-Cys residue modified with a caspase-3-responsive sequence (DEVD), a GSH-cleavable disulfide bond, and an ^{18}F-labeled zwitterionic organotrifluoroborate ($[^{18}F]$-$AMBF_3$) moiety. After the proteolysis action by caspase-3 and GSH-mediated reduction, the intramolecular cyclization gives rise to **16-MC**, then the hydrophobic interaction and π-π stacking promote the formation of NIR absorptive and radioactive nanoparticles (**16NPs**) through self-assembly. In viable tumor cells, the inactive pro-caspase-3 can not cleave the DEVD sequence, and the GSH cleaved probe could be cleared away from the tumor tissue, leading to low PET and PA signals (Figure 7D). However, in the apoptotic tumor cells, the pro-caspase-3 transforms into caspase-3, which could further induce the macrocyclization and self-assembly of **16-1** into **16-NPs**. The self-assembly behavior could significantly prolong the retention time of **16-NPs**, enhancing the PA signals and PET contrast. The **16-1** can be used to image the tumor apoptosis in a noninvasive PA/PET manner, providing a platform to early evaluate the therapeutic efficacy of antitumor drugs in vivo.

3. Conclusion and Outlook

This work discusses the chemical reactions induced peptide self-assembly in living systems triggered by the tumor microenvironment. The overexpressed enzymes at tumor-specific sites such as ALP, CES, CTSB, ACP, MMP-2, caspase-3, acidic microenvironment, and redox species with high levels could trigger the self-assembly of the peptide at the tumor-specific chemical environment, resulting in the better selectivity and killing effect than the traditional methods towards cancer cells. Furthermore, combining two or more stimuli could enhance the selectivity and efficiency of cancer therapy and reduce the side effects on normal cells. The chemical reactions induced peptide self-assembly exhibits great potential in the broader applications of cancer therapy, drug delivery, and biological diagnosis.

Although many exciting achievements have been achieved in the field of chemical reactions induced self-assembly, some critical challenges remain to be solved. On the one hand, we still need to discover new stimuli to trigger peptide self-assembly in living cells and in vivo, and the sensitivity of triggers should also be improved. In addition, achieving the peptide self-assembly in a spatiotemporal manner remains a significant challenge. On the other hand, we also need to develop advanced technologies and characterizations to probe the peptide self-assembly process in the complicated cellular environment and human body. The development of chemical reactions-induced peptide self-assembly is underway.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: cancer therapy · chemical reactions · enzyme · peptide · self-assembly

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