

Review

Peptide-modulated self-assembly as a versatile strategy for tumor supramolecular nanotheranostics

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Received: 2018.11.26; Accepted: 2019.03.09; Published: 2019.05.18

Abstract

Advances in supramolecular self-assembly have promoted the development of theranostics, the combination of both therapeutic and diagnostic functions in a single nanoplatform, which is closely associated with antitumor applications and has shown promising potential in personalized medicine. Peptide-modulated self-assembly serves as a versatile strategy for tumor supramolecular nanotheranostics possessing controllability, programmability, functionality and biosafety, thus promoting the translation of nanotheranostics from bench to bedside. In this review, we will focus on the self-assembly of peptide-photosensitizers and peptide-drugs as well as multicomponent cooperative self-assembly for the fabrication of nanotheranostics that integrate diagnosis and therapeutics for antitumor applications. Emphasis will be placed on building block design, interaction strategies and the potential relationships between their structures and properties, aiming to increase understanding of the critical role of peptide-modulated self-assembly in advancing antitumor supramolecular nanotheranostics.

Key words: peptides; self-assembly; intermolecular interactions; nanotheranostics; cancer

Introduction

Nanotheranostics that combine therapeutic effects and diagnostic capabilities in a single nanoplatform have attracted increasing attention in recent years because of their potential for application in personalized medicine, including tumor-related treatments [1-6]. Such smart integrated protocols are advantageous over discrete steps of diagnosis and therapy because they can provide real-time readouts of lesion responses and thus, in turn, help to guide therapy in an adaptive, personal and precise manner. Nanotechnology, exploited as emerging techniques for the fabrication of nanomaterials, has produced a large arsenal of nanomaterials with diversified capabilities in diagnosis and therapy [7-11].

In nature, the self-assembly or organization of biomolecules into intricate and functional units in living organisms provides a rationale to construct supramolecular structures that harness the power of

multiple noncovalent forces, including electrostatic forces, van der Waals forces, π interactions, hydrophobic interactions and coordination interactions [12-14]. These attractive forces are typically much weaker than covalent bonds, and these interactions occur in a dynamic and reversible manner, thus allowing self-assemblies to adapt to physiological conditions and maximize their biological functions [15-17]. In particular, peptides, as the fundamental components of proteins, show superior characteristics to those of existing assembly motifs as building blocks for the fabrication of nanotheranostics. These advantages include inherent biological origin, pharmacological safety, structural programmability, versatile functionality and easy availability. As a consequence, peptide self-assembly can be exploited as a fabrication strategy for creating various nanoscale theranostics [18-20]. Through

peptide-regulated cooperative noncovalent interactions, diagnostic and/or therapeutic agents are combined to form an integrated nanoplatform. The unique significance of peptide modulation lies in three aspects. (i) With respect to structure, varying the sequences of peptides consisting of amino acids or modified peptides with regulatory groups can yield various nanostructures because of the diverse noncovalent interactions involved. For example, hydrophobic interactions play a predominant role in micelle formation [21], while hydrogen bonds can induce directional growth into nanofibers in a thermodynamically favorable pathway [22]. Alternatively, manipulating the kinetic parameters (pH, temperature, counter ions, concentrations, solvents) [23-25] can also trap assembly structures in a metastable state with different morphologies. Therefore, desired nanostructures can be obtained by thermodynamic control or kinetic control. (ii) With respect to function, the coassembly of peptides and other bioactive components is a facile and typical strategy, where two or more types of building blocks interact with each other through synergetic noncovalent interactions to form nanostructures. Simultaneously, the dynamic nature of noncovalent interactions can be easily exploited to achieve the targeted release of bioactive components in response to a specific lesion microenvironment. For example, coassembly involving metal coordination integrated the dual properties of robust stability in circulation and smart responsiveness to tumor lesions, thereby enhancing the therapeutic effect [26-29]. Furthermore, some metal ions with intrinsic magnetic or radioactive properties could also be incorporated into coassemblies to perform unique imaging functions [29-31]. Another strategy is to synthesize peptide conjugates due to the feasibility of conjugation chemistry [32-36], which allows the design of specific building blocks, including peptide-photosensitizer and peptide-chemodrug blocks, followed by self-assembly into nanostructures with relatively high covalent stability and high loading efficiency. In addition, stimuli-responsive [37-39], circulation-extending [40] and target-binding [41, 42] sites can be flexibly introduced to increase the target-to-background contrast in imaging application and simultaneously improve the local concentration of therapeutic compounds at the target of interest, with the purpose of improving the therapeutic index. (iii) Last but not least, structural regulation facilitates function amplification. In particular, the enhanced permeability and retention effect (EPR) occurs due to the increased permeability of the blood vessels and dysfunctional lymphatic drainage in solid tumors during the systemic circulation of the combined

molecules [43], whereas this effect is not attainable by individual building blocks due to their quick excretion by the reticuloendothelial system (RES). In light of these advantages, peptide-modulated self-assembly serves as a flexible and versatile toolbox for the construction of tumor supramolecular nanotheranostics, highlighting the potential to predict the circulation and accumulation of agents at tumor sites, indicate uptake and achieve the sustainable release of drug molecules thereby improving therapeutic outcomes. However, elaboration of the structure-property relationship, including the manipulation of noncovalent interactions towards structural and functional regulation, is still a challenge [44, 45]. Therefore, a systematic summary of regulatory principles, properties and applications concerning peptide self-assembly is necessary for the future development of cancer nanotheranostics.

In this review, we mainly focus on three assembly strategies: (1) the self-assembly of peptide-photosensitizers, (2) the self-assembly of peptide-drugs and (3) multicomponent cooperative self-assembly for the fabrication of nanotheranostics that integrate diagnosis and therapeutics for antitumor application (Figure 1). Emphasis will be placed on building block design, intermolecular interactions and the potential relationships between their structures and properties, aiming to elucidate the critical role of peptide-modulated self-assembly in advancing antitumor supramolecular nanotheranostics. Representative studies based on above strategies are included, which will open promising prospects in the precise design of phototherapeutic agents, chemotherapeutic agents and multi-modal imaging-guided nanotheranostics for highly efficient cancer therapy. Finally, we will outline the challenges and potential of the use of nanotheranostics to promote optimized treatment outcomes.

1. Peptide-photosensitizer assembly strategies

Owing to their spatiotemporal selectivity and noninvasive nature, phototherapies, including photodynamic therapy (PDT) and photothermal therapy (PTT), have become promising approaches in the application of antitumor theranostics [46-48]. Overall, both modes rely on the specific delivery of photosensitizers to tumor sites, followed by photoirradiation to excite the photosensitizers. The excited photosensitizer decays back to the ground state through three main pathways: photon emission (fluorescence), intersystem crossing (energy transfer to generate reactive oxygen species, PDT) and nonradiative relaxation (photothermal conversion,

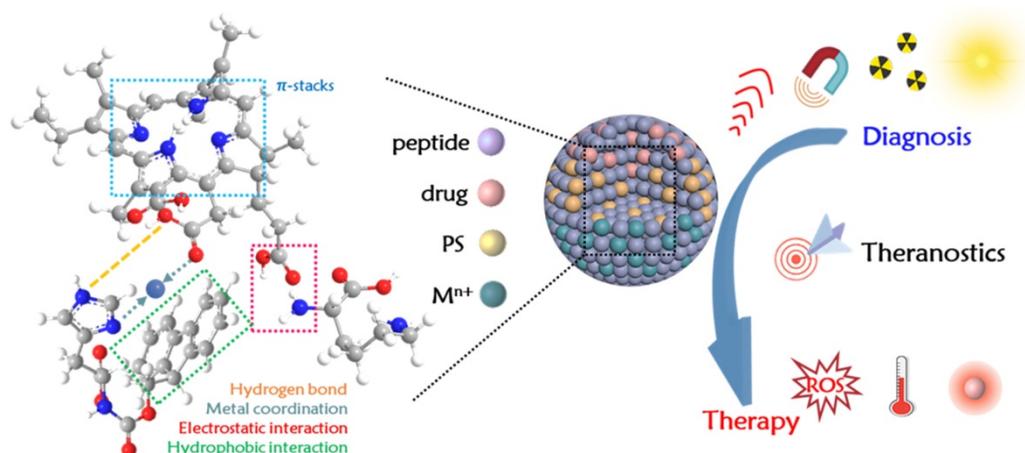


Figure 1. Schematic illustration of peptide-modulated self-assembly (peptide-photosensitizers; peptide-drugs; multicomponent cooperative self-assembly) as a versatile strategy for tumor supramolecular nanotheranostics.

PTT). Based on these three pathways, the requirements of the photosensitizer state for PDT and PTT differ. For PDT, the encapsulated or coassembled photosensitizers should be quickly released as monomers at the site of interest to perform ROS generation [49, 50]. For PTT, however, the photosensitizer should be maintained in an aggregated state since close stacking efficiently quenches fluorescence and intersystem crossing, promoting the dissipation of the absorbed energy into the lesion through nonradiative relaxation, i.e., photothermal conversion [51, 52].

1.1 Peptide-modulated self-assemblies for PDT

The wide application of PDT in clinical use is barred by the high hydrophobicity of photosensitizers, as the resulting ordered or disordered aggregation reduces their bioavailability and accumulation at lesions, which has instigated the development of supramolecular materials that improve their pharmacokinetics and tumor targeting [32]. Peptide-modulated self-assembly nanotheranostics developed as a promising approach to increase the solubility of photosensitizers in a nanostructure, extend their blood circulation time, and thus improve their specific accumulation at tumor sites. Intriguingly, photosensitizer molecules sometimes participate in the process of peptide self-assembly for the formation of nanotheranostics [53]. For example, porphyrins are organic heterocyclic macrocycles with photophysical properties well suited for PDT and fluorescence imaging [52]. Importantly, their intrinsic hydrophobic properties and π groups can provide noncovalent interactions to promote coassembly with peptides and thereby afford the final nanostructures [54-56]. Our group [55] previously demonstrated that short peptides of a diphenylalanine derivative, H-Phe-Phe-NH₂·HCl (CDP), and an amino acid

derivative, 9-fluorenylmethoxycarbonyl-L-lysine (Fmoc-L-Lys), can both coassemble with chlorin e6 (Ce6) through π -stacks, hydrophobic effects and electrostatic interactions. The resulting nanoparticles with superior features of high loading efficiency and smart drug release can lead to fluorescence imaging-guided PDT.

To precisely release photosensitizers at the site of interest (tumor), a highly appreciated strategy has emerged: the incorporation of interaction sites or functional groups that can respond to the tumor microenvironment (low pH of 6.0-7.0 [57, 58], heightened glutathione (GSH) level [59, 61], overexpressed enzymes [62, 63] and biomarkers [62, 64]) to achieve controlled photosensitizer activation at tumor sites rather than normal tissues [65, 66]. Major mechanisms of stimuli sensing involve breaking the assembly-disassembly balance between noncovalent interactions. However, complex physiological components, dilution by body fluids and degradation by enzymes may cause the premature disassembly of nanotheranostics before they reach the tumor sites. To address this concern, a novel method has recently been suggested: coordination assembly. Natural self-organized proteins in living organisms, such as metalloproteins, are formed by metal ions and other organic cofactors through coordination interactions [67], where metal ions not only improve stability and mechanical strength but also play a significant role in regulating biological functions [68]. With natural examples as a guide, the metal-triggered self-assembly of peptides is a promising direction for constructing well-defined conformations or nanostructures [69-71]. Above all, the strategy meets the requirements of robust circulation in physiological environments and stimuli-responsive photosensitizer release, as metal ions can coordinate with competing ligands at high concentrations within tumor tissues.

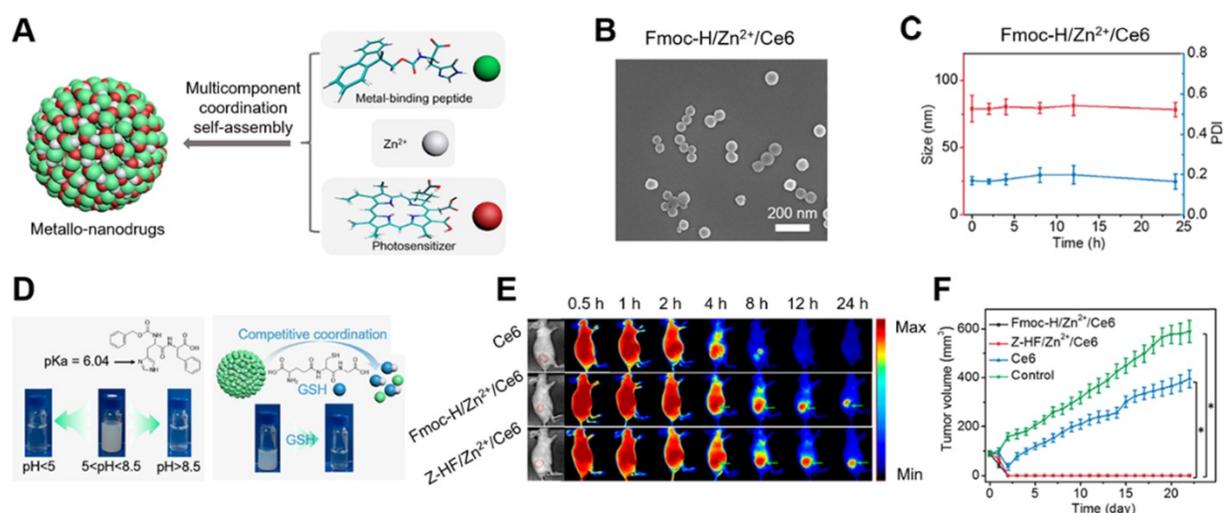


Figure 2. (A) Construction of metallo-nanodrugs through multicomponent (small peptides, photosensitizers, zinc ions) coordination self-assembly. (B) SEM image of the metallo-nanodrugs Fmoc-H/Zn²⁺/Ce6. (C) Size and PDI of metallo-nanodrug under conditions mimicking physiological circulation. (D) Schematic illustration of ultrasensitive responsiveness to pH and GSH change. (E) Fluorescence images showing the preferential accumulation of metallo-nanodrugs at tumor sites in contrast to unencapsulated photosensitizers. (F) Tumor growth profiles after PDT. Adapted with permission from Ref [26]. Copyright 2018 American Chemical Society.

Hemoglobin organically integrates histidine residues and porphyrin derivatives through cooperative coordination. Natural examples inspired us to engineer metallo-nanodrug theranostics by a multicomponent coordination self-assembly fabrication method [26]. Histidine-containing dipeptides (N-benzyloxycarbonyl-L-histidine-L-phenylalanine, Z-HF) or amphiphilic histidine derivatives (fluorenylmethoxycarbonyl-L-histidine, Fmoc-H) are selected as starting building blocks for coordination self-assembly. Spherical metallo-nanodrugs (~80 nm) can be readily regulated by zinc ion coordination with peptides and chlorin e6 (Ce6) combined with other noncovalent interactions (Figure 2A, B). Such coordination interactions exhibit dual properties in theranostic applications. On the one hand, the intermolecular coordination forces are nearly strong as covalent bonds, and such stable interactions can resist dilution or decomposition under normal physiological conditions (Figure 2C), in which Ce6 molecules exist in an aggregated state with a self-quenching effect, protecting their photosensitization, and thus exhibit prolonged blood circulation and improved tumor accumulation. On the other hand, the dynamic nature of coordination forces and noncovalent interactions is susceptible to the tumor-related microenvironment. Decreased pH and increased GSH levels jointly promote the disassembly of metallo-nanodrugs due to the carboxyl protonation and competitive coordination of zinc ions in the presence of GSH (Figure 2D). Such ultrasensitive responsiveness can free Ce6 and activate both fluorescence feedback and ROS generation within tumor cells. The drug distribution can be traced by *in vivo* fluorescence imaging and provide therapeutic

windows at 4 h post injection (Figure 2E). Under the guidance of the therapeutic window, effective molecular Ce6 drug concentration can promote PDT efficacy to eradicate tumors (Figure 2F). Recently, another assembly strategy involving metal coordination combined with other noncovalent forces was applied in host/guest chemistry by Granja and coworkers [72]. The host structure is composed of two self-complementary α,γ -cyclic peptides, which bear a Zn porphyrin cap for the selective recognition of the guest. The two components of the host structure are linked *via* two dynamic covalent bonds. Combined with hydrogen bonding, this linkage allows the two host molecules to self-assemble into a capsule structure. The metal ion coordination can recognize guest molecules (bipyridine) to form a sandwich complex structure. The hydrolysis of the hydrazones allows the reversible release of the encapsulated guest molecules due to the disruption of the capsule structure, suggesting the potential of these molecules in drug delivery vehicles, molecular machines and tumor theranostics.

As a distinct alternative tool, aggregation-induced emission (AIE) based building blocks for fabrication of nanostructures has gained prominence in the field of cancer theranostics [73-75]. The building blocks are non-emissive in the molecularly dissolved state while induced to emit fluorescence in the aggregation state mainly due to the restriction of intramolecular rotation (RIR). In sharp contrast to conventional nanotheranostics based on photosensitizers with aggregation-caused quenching (ACQ) effect, the AIE-based nanotheranostics possess superb advantages, such as high stability and enhanced therapeutic effect, particularly when

incorporation of smart activation strategy of photosensitizers [76-78]. For example, Liu and coworkers [76] designed a bioprobe for a target-specific light-up imaging and activatable photodynamic therapy. The bioprobe consists of four parts: Tetraphenylethene derivative (TPECM) functions as an imaging and photosensitizer reagent. Peptide sequence Gly-Phe-Leu-Gly (GFLG) can be responsive to cathepsin B. Tripeptide Asp-Asp-Asp (DDD) is a hydrophilic linker while increases the hydrophilicity of probe. A cyclic Arg-Gly-Asp (cRGD) moiety was incorporated for targeting tumor cells with overexpression of $\alpha_v\beta_3$ integrin. In aqueous media, the probe is nearly nonfluorescent and can rarely generate ROS owing to intramolecular motions. Once the bioprobe was selectively taken up by tumor cells, GFLG will be cleaved by cathepsin B and thus increase the hydrophobic interactions of TPECM for the formation of aggregation, leading to enhanced fluorescence emission and activated ROS generation for image-guided PDT. Furthermore, they developed another AIE-based probe, TPETP-SS-DEVD-TPS-cRGD [77], among which tetraphenylethene thiophene (TPETP) is a photosensitizer for PDT and peptide sequence Asp-Glu-Val-Asp (DEVD) is responsive to caspase-3/-7 for indicating cell apoptosis. In addition, cRGD and -S-S- are used as targeting ligand and linker, respectively, to realize dually-targeted activatable PDT. More importantly, when the probes were excited through 405 nm laser, the incorporated TPETP is red-emissive, while hydrophobic TPS showed intense green fluorescence after cleaved by caspases and therefore to report the therapeutic effect. Such a strategy based on distinct fluorescence turn-on signal is more favorable in complicated physiological conditions. Moreover, the activatable strategy can

expand to other biological interactions, such as specific YSA peptide (YSAYPDSVPMMS)-EphA2 protein interaction [78].

1.2 Peptide-modulated self-assemblies for PTT

The use of peptide-photosensitizer conjugates as building blocks for nanotheranostic construction has attracted significant attention in recent years owing to their facile synthesis and their simple but compact structural architecture. Significantly, the unique photothermal efficiency can be enhanced by the high sensitizer loading density, which markedly reduces the intermolecular distance of the photosensitizers and thereby inhibits fluorescence and intersystem crossing pathways, opening an avenue for PTT. Light absorption by molecules can create a thermally induced pressure jump that produces ultrasonic waves, which are detected by acoustic detectors for imaging (photoacoustic imaging, PA) with deep tissue penetration and high spatial resolution [79, 80]. Therefore, PA imaging-guide PTT expanded the repertoire of imaging and therapeutic modalities in which porphyrin agents can engage [81]. In addition, compared to the modality of PDT, PTT shows advantages in the treatment of hypoxic tumors because of its oxygen independence.

Zheng and coworkers [82] developed porphyrinsomes, nanovesicles formed from self-assembled porphyrin bilayers that showed photothermal conversion property. Therefore, the obtained porphyrinsomes were capable of visualization of lymphatic systems through PA imaging. After intravenous injection, such porphyrinsomes accumulated in tumors and revealed laser irradiation-induced photothermal tumor ablation. However, lipid bilayers are generally considered

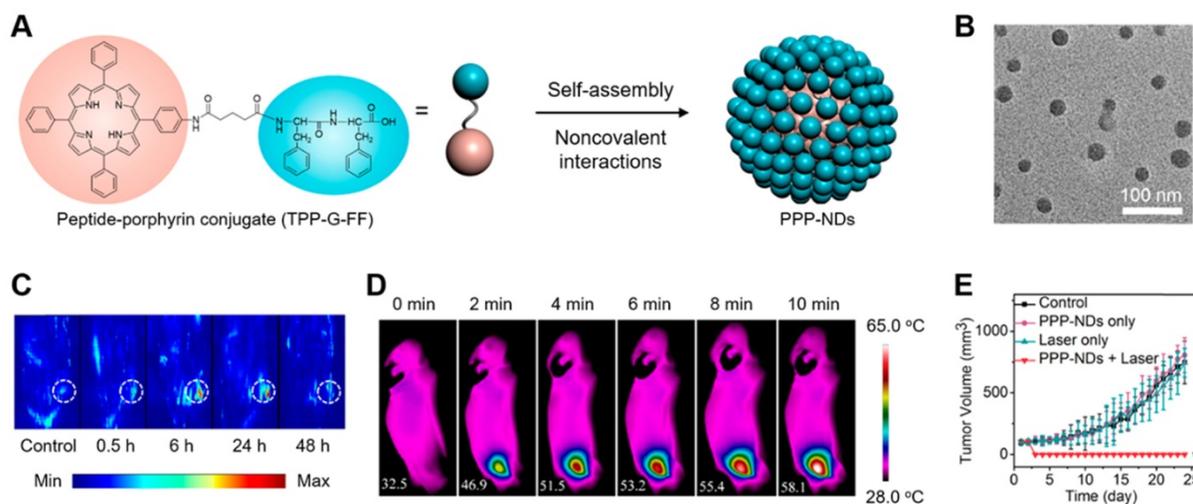


Figure 3. (A) Construction of photothermal peptide-porphyrin nanodots (PPP-NDs) by peptide-porphyrin conjugate (TPP-G-FF) self-assembly. (B) TEM image of PPP-NDs. (C) PA images of mice over time after the intravenous injection of PPP-NDs (D) IR thermal images of intravenous PPP-NDs injected into mice under continuous irradiation. (E) Tumor growth profiles after PTT. Adapted with permission from Ref [83]. Copyright 2017 American Chemical Society.

unstable structures in blood circulation and in storage. In this regard, our group [83] designed and synthesized a diphenylalanine peptide-porphyrin conjugate (TPP-G-FF) (Figure 3A). Upon the incorporation of FF, which is capable of tuning self-assembly, the amphiphilic structures form strong π -stacking and hydrogen bonding interactions and thus assemble into regular nanodots (PPP-NDs) with a diameter of 25 ± 10 nm (Figure 3B). Supramolecular aggregation resulted in complete fluorescence quenching and the inhibition of ROS generation, leading to the absorbed light energy to dissipate thermally with high conversion efficiency (54.2%). Notably, the photothermal conversion of PPP-NDs can be visualized through PA imaging techniques *in vivo*. The imaging results demonstrated that the optimal tumoral accumulation and retention of PPP-NDs was 24 h (Figure 3C). In this therapeutic window, IR thermal imaging indicated that the mean temperature within 10 min at the tumor sites increased to 58.1 °C upon laser irradiation (Figure 3D). The elevated temperature induced tumor cell necrosis irreversibly, suggesting robust PTT potency towards antitumor therapy (Figure 3E).

In principle, such peptide-modulated porphyrin-based self-aggregates for nanotheranostics can also be applied to other photosensitizers, for example, phthalocyanine. Yoon and coworkers [84] designed a “one-for-all” nanomaterial, NanoPcTB, assembled from phthalocyanine conjugates, in which the photoactivity of phthalocyanine can be switched from PTT to PDT, and the imaging mode can simultaneously be changed from PA imaging to fluorescence imaging by biotin receptor-assisted partial disassembly at tumor sites. Spatiotemporally integrated phototherapy guided by the dual imaging mode can eradicate tumors thoroughly. Alternatively, a more contemporary approach focuses on tailoring peptide sequences to amplify the final therapeutic effect. The conjugation of functional sequences, including antibodies, receptors, and some chimeric stimuli-responsive groups, to peptides is being explored [85-89]. For example, a chimeric peptide sensitive to both pH and matrix metalloproteinase-2 (MMP-2), Fmoc-12-aminododecanoic acid- H_8R_8 -PLGVR-PEG₈ (Fmoc-ADDA- H_8R_8 -PLGVR-PEG₈), developed by Zhang and coworkers [90] can be used to codeliver the photosensitizer protoporphyrin IX (PpIX) and plasmid DNA to MMP-2-rich tumor cells simultaneously. The chimeric peptides undergo hydrolysis of the PLGVR peptide sequence and exfoliation of PEG, with increasing positive charges, leading to cationic R_8 sequence exposure and preferential uptake by tumor cells. Furthermore, the decreased pH value in the endosomes leads to rapid

protonation of the peptide H_8 sequence and loss of its hydrophobic core, thereby releasing PpIX in the acidic endosomes. Moreover, a dual-stage light irradiation strategy was developed to realize the synergistic effect of drug and gene delivery. Upon short-term light irradiation, the “proton sponge” effect and the photochemical internalization effect can induce the endosomal escape of PpIX and DNA. Upon long-term irradiation, the synergistic efficacy of photodynamic and gene therapies was enhanced. As a result, chimeric peptide/PpIX/p53 complexes display significant inhibition of tumor growth.

2. Peptide-drug assembly strategies

Currently, chemotherapy is still one of the most effective ways for clinical treatment of cancer. Despite the clinical success of several chemotherapeutic drugs, it is still an unprecedented challenge to tailor the desired pharmacokinetics that allow chemotherapeutics to passively or actively target tumors while minimizing their adverse side effects. Over the past several decades, supramolecular nanotheranostics have emerged as an appealing approach to this goal, exhibiting improved solubility, prolonged circulation time and enhanced specific accumulation of chemodrugs in the tumor site owing to the EPR effect, thereby improving the overall therapeutic index [91]. Among a large number of research works, the chemodrug was passively encapsulated or absorbed in the nanostructure; this type of fixed “nanocarrier” protected the chemodrug molecules from degradative processes in the organism but resulted in limited, low drug payloads [92]. Peptides as building blocks possess multiple noncovalent interaction sites that can coassemble reciprocally with chemodrugs, presenting a more tunable and flexible way to increase drug loading. However, the intrinsic nature of noncovalent interactions could not resist harsh physiological conditions during *in vivo* application. Therefore, nanotheranostic designs that integrate flexible and high loading efficiencies as well as considerable stability are of interest. Two novel strategies aiming to solve the above problems are outlined below.

2.1 Metal coordination-driven self-assemblies for chemotherapy

Metal coordination-driven multicomponent self-assembly is a versatile strategy that can improve the circulation stability of chemodrugs and achieve targeted release. Our group [27] suggested that curcumin-based nanostructures can be fabricated through the coordination interactions of metal ions with metal-binding peptides or amino acids. Curcumin nanoparticles with well-defined, uniform

distributions were constructed from a histidine derivative (Fmoc-H), curcumin and zinc ions via cooperative coordination bonding together with other noncovalent interactions. Through control by kinetic parameters (tension coefficients of the solvent used to dissolve Fmoc-H) or dynamic parameters (concentration of curcumin), the size can be readily tuned from 80 nm to 180 nm (Figure 4A). The resultant nanodrugs possess high loading efficiency of curcumin (~52%) and protect it from degradation. Moreover, the dynamic ligand change or exchange in the tumor microenvironment conferred responsive release properties on the nanodrugs, therefore improving drug uptake by tumor cells. Fluorescence imaging indicated favorable circulation and accumulation in tumor sites. Collectively, these features increased the stability of curcumin and simultaneously improved tumor accumulation, resulting in enhanced antitumor therapy. The coordination-driven multicomponent self-assembly opens an avenue of chemodrug use for antitumor therapy.

2.2 Peptide-conjugate self-assemblies for chemotherapy

The covalent modification is an alternative clinically proven strategy to devise peptide-drug conjugates for self-assembly with enhanced treatment efficacy [93, 94]. Peptide sequences containing stimuli-responsive groups can be incorporated on demand, which not only plays a significant role in drug release but also promotes the strategy formation of intracellular (*in vivo*) self-assembly. Intracellular self-assembly based on biocompatible condensation reaction [95-97], endogenous stimuli reconstruction upon the control of either pH, disulfide reduction or enzymatic cleavage [17, 98] has shown great potential in improving targeting cellular uptake of the drug molecules in chemotherapy.

Xu and coworkers [99-103] performed pioneering works on designing and constructing drug-peptide amphiphilic hydrogelators that can

self-assemble into supramolecular three-dimensional hydrogel networks. The dephosphorylation/phosphorylation cycle catalyzed by the alkaline phosphatase (ALP)/kinase switch has been applied to control self-assembly. For example [99], by introducing a phosphotyrosine into a Nap-Phe-Phe-Lys (PTX) hydrogelator, where Nap refers to a naphthalene moiety in order to increase the solubility or hydrophilicity but with no effect on the self-assembly property. Dephosphorylation occurred once the hydrogelator contacted the phosphatase enzyme; thus, the hydrophobicity was enhanced, further promoting spontaneous self-assembly *via* β -sheet interactions to form a hydrogel. Once the gel was formed, the PTX conjugates were sustainably released and exhibited effective cytotoxicity against HeLa cells. By comparison, Yang and coworkers [104, 105] recently provided a different approach to phosphorylation. The targeting tripeptide RGD with hydrophilic properties was conjugated to curcumin through a reducible disulfide bond linker to form a hydrogelator [104]. Due to the specific binding to integrin expressed on tumor cells, the localization of the hydrogelator was enhanced after systemic injection. After localization, the elevated intracellular GSH concentration reduced the disulfide bonds. Consequently, the RGD sequence was cleaved from the curcumin hydrogelator, increasing the hydrophobicity of building blocks and therefore allowing for their self-assembly spontaneously. The released curcumin showed obvious cytotoxic activity to tumor cells and inhibited tumor growth. Similarly, Ding and coworkers [106] designed another curcumin-peptide conjugate (Curcumin-FFE-CS-EE). By GSH reduction, the hydrophilic EE sequence was removed, leading to the formation of curcumin-based supramolecular nanofibers for use as radiosensitizers. Liang and coworkers [107, 108] design a precursor Cys(SET)-Glu-Tyr(H₂PO₃)-Phe-Phe-Gly-CBT that is responsive to extracellular ALP and intracellular GSH in tumor site for yielding cyclic amphiphilic 2D building blocks. Interestingly, the precursor

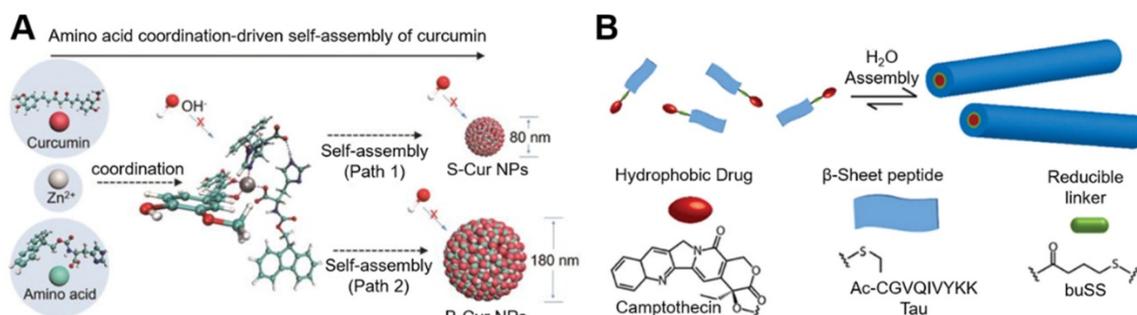


Figure 4. (A) Curcumin nanoagents based on amino acid coordination-driven self-assembly. Adapted with permission from Ref [27]. Copyright 2018 Wiley VCH. (B) Illustration of a CPT-based drug amphiphile that can spontaneously self-assemble into nanofibers. Adapted with permission from Ref [110]. Copyright 2013 American Chemical Society.

molecules cannot enter the cells, but the converted cyclic amphiphilic 2D molecules can be efficiently internalized by cells and locally self-assemble into the nanofibers with enhanced mechanical strength, highlighting great potential of smart nanotheranostics design.

Cui and coworkers [33, 53, 109, 110] demonstrated the rational design of an amphiphilic peptide-drug conjugate (“prodrug”) that can form a variety of nanostructures. Intriguingly, the drug itself constitutes one of these two domains, most commonly the hydrophobic, and serves a structural role in addition to eliciting a therapeutic effect. Such conjugated peptide-drug prodrugs with a suitable hydrophilic-hydrophobic balance can self-assemble into well-defined supramolecular nanostructures in aqueous solution. For example [110], the hydrophobic drug camptothecin (CPT) was conjugated to a β -sheet-forming peptide sequence derived from the Tau protein through a reducible disulfonylbutyrate linker (buSS) (Figure 4B). In this design, the drug content can be precisely controlled by the number of CPT molecules, one, two or four, and the resultant drug loading can be 23%, 31%, and 38%, respectively. Moreover, the morphology can also be tuned by the number of branching points for attaching CPT molecules. Nanofilament to nanotube transitions can be tuned as a function of drug content since the hydrophobicity and possible π - π associative interactions among CPT inter- and intramolecular units were enhanced. This stable nanostructure sequestered CPT molecules in its core. Notably, biodegradable linkers offered responsiveness towards the external environment to release CPT sustainably and further increase the antitumor efficacy.

To maximize the effectiveness of chemotherapy, metal coordination and peptide conjugation can be flexibly combined. Cisplatin-based metal ions have been also reported to be capable of forming complexes with peptides containing carboxylic acid groups. Yang and coworkers [111] choose 10-hydroxycamptothecin (HCPT) and cisplatin to construct the dual anticancer drug assemblies. HCPT was firstly conjugated with peptide FFERGD to form amphiphilic building blocks, HCPT-peptide (*HP*). In order to assist *HP* entering into the cellular nucleus, cisplatin coordination was introduced to complex with *HP*. By tailoring the molar ratio of cisplatin/*HP* from 1 to 1.5, two different complexes were obtained and further self-assembled into long nanofibers (10-15 nm) and nanoparticles (20-25 nm), respectively. The nanomaterials play a role of “Trojan Horse” that transported dual anticancer drugs across the cell and nucleus membranes, showing synergistic inhibition effect to cancer cells.

Additionally, some active targeting groups, for example, surface markers (antigen or ligands), should be considered for peptide-drug conjugate design to enhance therapeutic results. The binding of ligands to receptors may cause receptor-mediated multivalent interactions to promote the internalization and release of drug molecules, alleviating the random diffusion of targeting drug molecules and thereby reversing multiple-drug resistance, which often causes chemotherapy treatments to fail [112, 113]. Moreover, conjugating peptides with functional groups targeting the tumor vasculature or tumor microenvironment as well as introducing imaging groups may also be necessary [114].

3. Multicomponent cooperative self-assembly strategies

Tumor heterogeneity and complex bionanostructure interactions have a considerable, underappreciated impact on treatment efficacy [57]. Consequently, it is reasonable to choose and explore a multicomponent cooperative self-assembly strategy to integrate manifold imaging techniques and multiple therapies into a single nanoplatform, thereby gaining mutually confirmative information on trafficking pathway and kinetics of delivery and accurately monitor therapeutic outcomes, an approach that shows great potential in clinical use [115].

Before initiating tumor treatment, it is essential to perform imaging to understand the tumoral biological information. In general, different imaging modalities have advantages and disadvantages that define their use in visualizing the genesis and development of lesions. Thus, the selected imaging modality should be suitable to reveal the dynamic features of tumors. Highly sensitive imaging techniques, including singlephoton emission computed tomography (SPECT), positron emission tomography (PET), and optical imaging, are being used to monitor tumor cell biology, tumor burden, tumor progression and metastasis, but they lack anatomical information to localize the signal, which is better provided by magnetic resonance imaging (MRI) and computed tomography (CT) [29, 30, 116]. Comprehensive imaging modalities can guide the field of nanotheranostics towards an era of more effective and personalized treatment.

3.1 Single mode imaging-guided therapy

As an example, our group [28] has designed a multicomponent coordination nanotheranostic platform by mixing 9-fluorenylmethoxycarbonyl-L-histidine (Fmoc-L-L), a photosensitizer (Chlorin e6, Ce6), and Mn^{2+} in aqueous solution to form Fmoc-L-L/ Mn^{2+} nanoparticles (FMNPs) driven by

coordination and other noncovalent interactions (hydrophobic interaction, π - π stacking) (Figure 5A). Carboxyl groups involved in amino acid coordination with Mn^{2+} play a significant role in improving the stability of the final nanostructure. More importantly, introducing Mn^{2+} forced the FMNPs to act as an MRI agent. The resulting minimalist nanoplatform showed smart responsiveness within tumor tissue, restoring Ce6 photosensitization capability to generate ROS for PDT and enhancing Mn^{2+} retention for MRI due to stronger binding with GSH. This integrated fluorescence imaging with MRI combined the merits of the respective qualitative and quantitative information of both methods and provided feedback regarding the therapeutic efficacy. At 4 h post injection, PDT was performed under the guidance of fluorescence imaging (Figure 5B) and MRI (Figure 5C). In addition, therapeutic performance was observed in the process of tumor ablation within three days.

3.2 Dual/multiple mode imaging-guided therapy

Focusing on tumor-targeted drug delivery, PET/MRI dual-modality tumor imaging was achieved by using arginine-glycine-aspartic (RGD)-conjugated radiolabeled iron oxide (IO) nanoparticles fabricated by Chen and coworkers [117]. PET and MRI have already been used in clinical practice. Cyclic RGD peptide modification promotes the selective bonding of nanoparticles to tumor blood vessels (via $\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$ integrin receptors overexpressed by activated endothelial cells), and the nanoparticles were further modified with DOTA to enable ^{64}Cu complexation and simultaneous PET-MRI monitoring (Figure 5D). PASP-IO nanoparticles with a

core size of 5 nm and a hydrodynamic diameter of approximately 45 nm were made by a single-step reaction. The saturation magnetization of PASP-IO nanoparticles is approximately 117 emu/g of iron, and the measured r_2 and r_2^* are 105.5 and 165.5 (s mM) $^{-1}$, respectively. The accumulation of ^{64}Cu -DOTA-IO-RGD nanoprobe was found to start at 1 h post injection and reached a peak value at 4 h. In the case of nontargeted probes and in the blocking experiment, hardly any accumulation in tumors could be visualized (Figure 5E). T_2 -weighted MRI was conducted to confirm these findings (Figure 5F). As a complementary example [118], a new type of dual integrin $\alpha_v\beta_3$ and GRPR targeting radiotracer, ^{68}Ga -BBN-RGD, was fabricated by their group and used for PET/CT imaging of breast cancer, showing great potential in discerning primary breast cancers, axillary lymph node metastasis and distant metastases.

Significantly, various imaging and therapeutic modalities can be integrated flexibly through peptide-modulated self-assembly to optimize final therapeutic outcomes [119, 120]. Wang and coworkers [119] introduced a paradigm of manipulating T_1 - T_2 dual-modal MRI for tumor theranostics. A nanosystem of $(Fe_3O_4@SiO_2@mSiO_2/DOX-(Gd-DTPA)-PEG-RGE)$ NPs was presented, in which chemodrug DOX was loaded and the tumor-penetrating peptide RGERPPR (RGE) functioned to increase the cellular uptake and cytotoxicity of NPs, while Gd-DTPA served as the T_1 contrast agent and Fe_3O_4 as the T_2 contrast agent. *In vitro* and *in vivo* treatment results on U87MG cells showed that the NPs could be used as an effective platform for tumor theranostics. Chen and coworkers [120] conjugated a somatostatin receptor (SST)

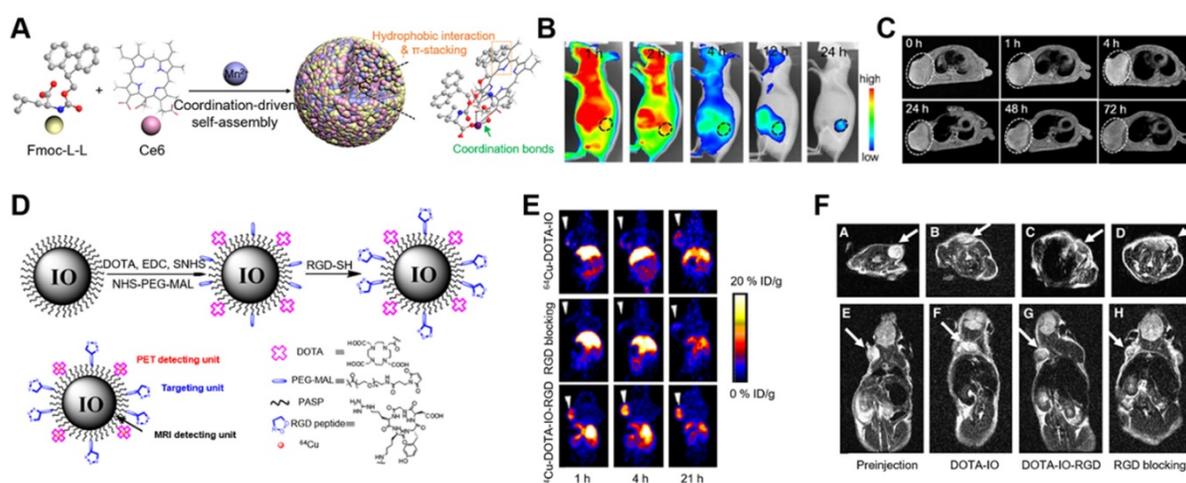


Figure 5. (A) Small amino acid- Mn^{2+} metal ion coordination-driven supramolecular self-assembly. (B) Fluorescence images of mice following the intravenous injection of FMCNPs. (C) T_1 -weighted MR images of mice following the intravenous injection of FMCNPs. Adapted with permission from Ref [28]. Copyright 2018 American Chemical Society. (D) Synthesis of PET/MRI dual-functional probe DOTA-IO-RGD. (E) PET images of mice after the injection of ^{64}Cu -labeled nanoprobe. (F) T_2 -weighted MR images of mice before and after the injection of nanoparticles. Adapted with permission from Ref [117]. Copyright 2008 Society of Nuclear Medicine, Inc.

peptide derivative (DOTA-octreotate), to an Evans blue analog (EB) that can specifically bind to circulating serum albumin. The resulting molecule was used to chelate ^{86}Y and ^{90}Y , which play roles in PET imaging and radiotherapy, respectively. The imaging capability and the radiotherapeutic efficacy were demonstrated to be effective for the treatment of SSTR2-containing tumors.

Conclusion

In summary, we have provided an overview of recent fascinating developments in the area of peptide-modulated self-assembly as a versatile strategy for tumor supramolecular nanotheranostics. Peptides function as molecular building units or biologically active compounds to build supramolecular nanostructures owing to their molecular design versatility, tunable biocompatibility and biodegradability. The resultant peptide-photosensitizers, peptide-drugs and multicomponent cooperative self-assemblies can be regulated in terms of composition, noncovalent interactions and physiochemical properties. The obtained nanotheranostic platforms demonstrated precise selectivity, smart adaptability, and multifunctionality towards antitumor theranostics, which amplified the therapeutic functions, providing prolonged blood circulation, enhanced tumor accumulation, and increased sensitivity to lesion response.

Although considerable success has been achieved in supramolecular nanotheranostics, the main challenge in cancer therapy stems from the heterogeneity and complexity of the tumor entity and microenvironment, which cause incomplete or unpredicted interactions (trafficking, internalization, penetration, distribution, fluid pressure) between nanotheranostics and tumor cells *in vivo* [121]. A deep understanding of tumor biology combined with noninvasive imaging techniques with fast response and ultrahigh resolution at the single-cell level will elucidate complex bionanotheranostic interactions. Moreover, most supramolecular nanotheranostics are delivered intravenously for systemic transport to tumors through defective tumor vessels and impaired lymphatics in the tissue: the enhanced permeability and retention (EPR effect). Such transportation is a fundamental principle but is substantially considered to be oversimplified [122] because it is increasingly clear that EPR varies among patients and tumor types, even within the same patient or the same tumor type over time [123]. Taking this point into consideration, the discovery of novel disease targets (overexpressed receptors) is necessary. Furthermore, even though satisfactory treatment outcomes have been achieved, the selection of animal models or tumor type models

in research, cannot predict the clinical results; thus, the translation of most supramolecular nanotheranostics in humans remains largely unexplored. Therefore, a series of systematic, personalized studies in clinical use should be pursued. Last but not least, despite the promising therapeutic results of nanotheranostics covered in this review, two critical aspects on peptide-based supramolecular systems should be highlighted: (1) although peptides are biologically-originated building blocks, tedious chemical modification in order to meet desired demands for theranostics may provoke unwanted side effects such as inflammation and immunogenicity; (2) stability is another major concern. Peptide self-assembly is in harness of noncovalent interactions and thus prone to disassemble in complicated physiological environment, leading to premature drug release, side effects to normal tissue, and reduced drug efficacy in the site of interest. Therefore, ideal nanotheranostics should be stable enough in blood circulation while be unstable once they reach to the therapeutic site. In this regard, smart strategies that can enhance fabrication interactions and simultaneously manipulate nanodrugs to be smart responsiveness for tumor microenvironment will be highly appreciated. Insights into the mechanism for supramolecular nanotheranostics regarding materials design and therapeutic efficacy should be one of research focuses [51]. All in all, advancing supramolecular nanotheranostics may promote the clinical translation of nanodrugs and theranostics from bench to bedside.

Acknowledgements

We acknowledge financial support from the National Natural Science Foundation of China (Project Nos. 21773248 and 21802144), the National Natural Science Fund BRICS STI Framework Programme (No. 51861145304) and Innovation Research Community Science Fund (No.21821005) as well as the Key Research Program of Frontier Sciences of the Chinese Academy of Sciences (CAS, Grant No. QYZDB-SSW-JSC034).

Competing Interests

The authors have declared that no competing interest exists.

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