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D-Amino acid-Containing Supramolecular Nanofibers for Potential Cancer Therapeutics

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Abstract

Nanostructures formed by peptides that self-assemble in water through non-covalent interactions have attracted considerable attention because peptides possess several unique advantages, such as modular design and easiness of synthesis, convenient modification with known functional motifs, good biocompatibility, low immunogenicity and toxicity, inherent biodegradability, and fast responses to a wide range of external stimuli. After about two decades of development, peptide-based supramolecular nanostructures have already shown great potentials in the fields of biomedicine. Among a range of biomedical applications, using such nanostructures for cancer therapy has attracted increased interests since cancer remains the major threat for human health. Comparing with L-peptides, nanostructures containing peptides made of D-amino acid (i.e., D-peptides) bear a unique advantage, biostability (i.e., resistance towards most of endogenous enzymes). The exploration of nanostructures containing D-amino acids, especially their biomedical applications, is still in its infancy. Herein we review the recent progress of D-amino acid-containing supramolecular nanofibers as an emerging class of biomaterials that exhibit unique features for the development of cancer therapeutics. In addition, we give a brief perspective about the challenges and promises in this research direction.

Graphical Abstract

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Chemical compounds studied in this article

10-Hydroxycamptothecin (PubChem CID: 97226); O-Phospho-L-tyrosine (PubChem CID: 30819); Paclitaxel (PubChem CID: 36314); Dapi (PubChem CID: 2954); Taurine (PubChem CID: 1123); 4-Chloro-7-nitrobenzofurazan (PubChem CID: 25043)

Keywords

D-amino acid; nanofibers; cancer therapeutics; D-peptide; system biology; multifaceted

1. Introduction

Cancer is among the leading causes of death worldwide. In 2012 there were 14 million new cases and 8.2 million cancer-related deaths worldwide [1]. To relieve the suffer and to improve the quality of life of the patients, scientists from many disciplines are working on inventing new drugs, improving the delivery of current drugs, screening new targets, and developing early diagnostic methods of cancer. We and others have focused on utilizing nanotechnology for novel self-delivery of anticancer medicines and, particularly, *in situ* molecular self-assembly, as a process, to kill cancer cells selectively.

Molecular self-assembly prevails in cellular processes. For example, the self-assembly of actins and tubulins generates nanostructures as cytoskeletons and self-assembly of DNA serves as storage for genetic information [2, 3]. These biological processes have inspired the use of principle of self-assembly for developing functional materials in many areas. To mimic those biological systems, researchers have utilized sugar derivatives [4-7], amino acid derivatives [8, 9], DNA [10, 11], lipids [12, 13], and peptides [6, 7, 14] as the building blocks to construct a wide-range of nanomaterials. Among these, self-assembling peptides have attracted increasing attentions in recent years due to their easiness in design and synthesis [15-19]. Moreover, their readily modifiable and known functional motifs, good biocompatibility, low immunogenicity, minimal toxicity, inherent biodegradability, and fast responses to external stimuli have resulted in the applications of self-assembling peptides in various biomedical applications, such as three dimensional cell culture [20-24], drug delivery [25-33], cancer therapy [34-37], immune boosting [38-41], regenerative medicine [20, 42-44], and detection of important analytes (e.g., enzymes, metal ions, bacteria) [4, 45-52]. In order to form nanostructures, a stimulus, such as heating-cooling cycle [53], sonication [54, 55], pH adjustment [22, 56, 57], assistance of organic solvent [58-60], light irradiation [61-63], chemical fuels [64, 65], as well as enzymatic reaction [66-69], usually is needed [70]. The formed nanostructures are nanofibers in most of cases, as often revealed by transmission electron microscopic (TEM) imaging[71]. For the applications of these nanofibers in the cancer therapy, the common strategy is to trap the hydrophobic drugs in the hydrophobic core of the nanofibers, which usually boost release at the first stage but still suffers from the low capacity of drug loading [25, 72]. An alternative strategy is to use prodrugs that consist of therapeutic agents and a known self-assembly motif as the building blocks to construct active biomaterials [27-31, 73-75]. Such nanobiomaterials, especially nanofibers formed by self-assembly of drug molecules may lead to superior active biomedical materials for improving solubility, increasing loading capacity, eliminating or reducing harmful excipients, conferring responsiveness to biological cues (e.g., enzyme, redox event, or pH change), and even enhanced activity [76]. These improvements usually lead to controlling drug release as well as enhancing blood circulation. In such systems, the self-assembly of the prodrug largely utilized naturally occurring L-amino acids (as summarized in several excellent reviews [31, 77, 78]). Despite the advances in this field,

certain issues, such as biostability of the nanofibers, selectivity against cancer cells, and efficacies, remain as challenges. One solution to solve these problems is to use D-amino acids to replace L-amino acids. Although rarely being used by human cells in protein synthesis, D-amino acids are vital to some living organism including bacteria and mammals [79, 80]. Inspired by these facts in nature, several labs have employed D-peptides to construct self-assembly systems that exhibit excellent biostability [81-87]. Here we review the recent progresses of supramolecular nanofibers made of D-peptides, an emerging class of biomaterials that exhibit unique properties for developing cancer therapeutics. First, we introduce the nanofibers of peptides containing D-amino acids acting as physical carrier for hydrophobic drugs or serving as the building blocks for covalently conjugating with the drugs. Second, we discuss the prion like nanofibers of dipeptides for selectively inhibiting cancer by interacting with cytoskeleton proteins. Third, we highlight spatiotemporal control of the formation of nanofibers on cancer cell surface via enzyme-instructed self-assembly (EISA) of D-tripeptides for cancer therapy. Fourth, we describe the strategy of introducing taurine to boost cellular uptake of small D-peptides and its application in enhancing activity of cisplatin against platinum-resistant ovarian cancer cells. Finally, we give our perspective about the challenges and promises in the applications of supramolecular nanostructures containing D-amino acids for biomedicine.

2. D-Amino acid-containing supramolecular nanofibers for drug delivery

2.1. Nanofibers formed by D-peptides for encapsulation of anticancer drugs

The hydrophobic domain in self-assembled nanofibers allows the incorporation of hydrophobic therapeutic agents, and the drug incorporated nanofibers can find applications for local and controllable release of the drug molecules through the degradation of the nanofibers. Several groups have already developed these systems for anticancer drug delivery both *in vitro* and *vivo*. The use of the nanofibers of D-peptides to enhance the solubility of hydrophobic drugs, especially anticancer drugs, is gaining more attentions in recent years. D-peptide based nanostructures not only serve as a carrier to control the payload ratio and release rate, but also increase the resistance against proteolysis *in vivo*. The work of nanofibers containing D-peptides has validated the long-term biostability of D-peptide nanofibers both *in vitro* and *in vivo* [88-90]. After the demonstration of capacity of the D-peptide nanofibers for controlled drug release by *in vivo* imaging of ^{125}I and ^{131}I tracers [81], the use of nanofibers consisting non-nature amino acids as carriers for hydrophobic drugs has emerged as a new direction of nanomedicine.

Despite of the advances of peptide nanofibers as potential candidates for cancer therapy [26, 72], their *in vivo* biocompatibility and stability received less evaluation, a drawback that hinders their practical applications [91-93]. Recently, Yang and co-workers systematically studied the *in vivo* stability, distribution, and toxicity of the peptide nanofibers of peptides made of L- or D-amino acids (i.e., enantiomers **1** and **2**) [82, 94]. Using hydroxycamptothecin (HCPT) as a model drug, they have shown the better selectivity and antitumor efficiency of the nanofibers containing D-amino acids versus L-amino acids. Specifically, they used L- and D-Nap-Gly-Phe-Phe-Tyr (Fig. 1A) as an efficient hydrogelator to connect a tumor targeting tripeptide (RGD) which is known to serve as a recognition

motif in multiple ligands for several different integrin[95]. They tested the stability of both D- and L-nanofibers in mouse blood plasma by CLSM and HPLC, which reveals that the nanofibers of the L-peptide degrade almost completely after 24 hours. On the contrary, the nanofibers of the D-peptide remain intact after 24 hours incubation with blood plasma. Furthermore, after the use of ^{125}I to label the tyrosine (Y) residue of the peptides, the biodistribution study (intravenous injection of the nanofibers) of the D- and L-nanofibers *in vivo* indicates that D- and L-nanofibers result in quite different biodistribution (Fig. 1B): the nanofibers of L-peptide lead to the highest radioactive signals in the stomach tissues, and the L-peptides mainly distribute in the long and small intestine; the nanofibers of D-peptide exhibit preferential accumulation of the D-peptide in liver tissue, kidney, and large intestine. Both the L- and D-peptides could quickly cleared from the body after 12 hours and exhibit little toxicity toward kidney and liver. They tested the ability of the nanofibers as a carrier of hydrophobic drugs for cancer therapy. As shown in Figure 2C, the nanofibers can encapsulate HCPT via hydrophobic interaction without using any organic solvent. The loading efficiency of HCPT in D- and L-peptide nanofibers are 66% and 73%, which improves the solubility of HCPT in aqueous solution by 46 and 54 times, respectively. TEM images also suggest that HCPT has a negligible effect on the morphology of nanofibers formed by the peptides. In addition, *in vivo* experiments suggest that the nanofibers of D-peptide has a better anticancer efficiency than its corresponding L-peptide (Fig. 2D). This work, as a comprehensive example of systematic study on the fate of the nanofibers of small peptides, including *in vivo* stability, distribution, toxicity, and anticancer efficiency, has provided useful insights for the development of smart drug carriers based on D-peptides.

2.2. Nanofibers formed by the conjugation of D-peptides and anticancer drugs

Besides non-covalent encapsulation of drugs into a delivery system, another strategy is to incorporate drugs covalently for generating responsive prodrugs. Because of their unique advantages, such as enhanced bioavailability and responsiveness to microenvironment of cancer, prodrugs are under active development [96-98]. Despite the advances of prodrugs in drug delivery, there are unmet challenges, such as limited blood circulation [99-101], low-specificity [102, 103], and the need of toxic excipients [104, 105]. To overcome these drawbacks that limit the further applications of prodrugs, self-assembled prodrugs, which serve as both the cargo and the carrier, have received considerable research attentions in recent years [31]. With the ability of self-assembly, these prodrugs form nanostructures like nanofibers, nanosheets, nanospheres for a sustained release in aqueous environment without compromising the bioactivity of parent drugs [27, 29, 30, 73, 74, 106, 107]. The observation of self-assembled vancomycin, which inhibits superbugs such as Vancomycin-Resistant Enterococci (VRE) [76], represents an earlier and exceptional example of self-assembly of drugs. This unexpected phenomenon has inspired self-assembly of prodrugs based on the conjugation of peptides and therapeutic drugs, which provides an ideal platform for developing self-delivery systems of various therapeutic agents (e.g., paclitaxel [27, 30, 74], HCPT [29, 108, 109], cisplatin [110, 111], doxorubicin [112-114], and gemcitabine [115]).

Compared with normal strategies for self-assembly, EISA is a versatile strategy because it confers biocompatibility, broad applicability, and most importantly, high degree of spatiotemporal control and selectivity [66], which is readily applicable to L-peptides. Unlike

L-peptides that usually are good substrates for proteolytic enzymes, D-peptides are poor substrates of enzymes, a perception that hinders the introduction of D-peptides for EISA. The first case of D-peptide serves as substrates of enzyme for self-assembly, however, reveals that D-peptides not only can serve as substrates of mammalian enzymes, but also can boost the selectivity of clinical drugs (e.g., a non-steroidal anti-inflammatory drug (NSAID)) [106]. Recent study shows that the conjugation of a D-peptide with paclitaxel results in better anticancer efficiency than its L-enantiomer [89]. According to an X-ray structure of alkaline phosphatase, phosphate group in D-peptide (i.e., Nap-^DF^DF^DK^Dp^DY), like its L-peptide counterpart, could reach to the active site of alkaline phosphatase (Fig. 2A). ³¹P NMR (Nuclear magnetic resonance) also suggests that the dephosphorylation rate of Nap-^DF^DF^DK^Dp^DY is similar with its L-enantiomer. The attachment of paclitaxel, an effective anticancer drug, to a D-peptide produces the precursor (**3a**). Acting as a prodrug with self-assembling ability, **3b**, at an optimal concentration, form nanofibers/hydrogel after alkali phosphatase (Fig. 2B) converts **3a** to **3b**. Cell assay (against HeLa cells) suggested that **3a** has similar activity as paclitaxel, but **3b** exhibits less cytotoxicity (Fig. 2C). The study of the *in vivo* activity of **3b** and its enantiomer in tumor bearing mouse model shows that both the nanofibers of the conjugates of paclitaxel with D- or L-peptides exhibit better antitumor efficacy than paclitaxel alone (Fig. 2D). Moreover, **3b** exhibits higher antitumor efficiency than its L-peptide counterpart. The conjugation of an environment sensitive fluorescence dye to the D-peptide affords a precursor/hydrogelator pair for imaging the self-assembly process in cellular level. These exploration may lead to an approach for imaging specific microenvironment of cancers (especially when cancer cells overexpress certain enzymes, such as placental alkaline phosphatase [116, 117], as tumor markers). These studies have demonstrated that D-peptides can serve as substrates of certain enzymes for biomedical applications. The nanofibers formed by D-peptides not only resist degradation of proteases, but also serve as the reservoir for long term drug delivery. These observations are supported by the works of other labs. A very recent work, by Yang et al., showed that D-peptide based nanofibers/hydrogel serve as the carrier of antigens for cancer therapy [118], further demonstrating the potentials of this molecular platform based on D-peptides. Considering the advantages of D-peptides, it is likely that future exploration will lead to more applications of D-peptides [119].

3. Nanostructure formed by short peptides selectively kill cancer cells

Self-assembly plays significant roles in almost all biological processes. But the unintended or unregulated self-assembly could be detrimental to human health, as the case of the aggregates of aberrant proteins to cause neurodegenerative diseases [120]. The intriguing inverse comorbidity between cancer and neurodegenerative diseases [121] suggests that molecular nanofibers should inhibit cancer cells. This notion, indeed, is supported by the use of the nanofibers formed by self-assembling peptides to inhibit cancer cells. For example, the nanofibers formed by NapFF (Fig. 3A, compound **4**) can selectivity inhibit the growth of glioblastoma cells by disrupting the dynamic of microtubules and tubulins [37]. As shown in Figure 3B, compound **4** can form nanofibers both in PBS buffer and cell culture medium. The formed nanofibers have more tendency to accumulate in cells than the unassembled monomers. Several complementary approaches, such as hydrogel-based pull down assay

[45, 122], tubulin polymerization assay [123], and PathScan apoptosis multi-target sandwich ELISA [124], have provided insights on the apoptotic mechanism of the cell death caused by the nanofibers of that short peptide. That is, after entering the cells via macropinocytosis, nanofibers of **4** interact with cell skeleton proteins (actin and tubulin) through non-covalent interaction, then activate the caspase-dependent signal pathway (Bad and p53), prevent cell mitosis, and inhibit cell proliferation (Fig. 3C) [125]. Moreover, a mice tumor model (Fig. 3D) demonstrates the antitumor efficiency of the nanofibers *in vivo* [126]. In addition, the nanofibers of the relevant D-peptide, **5** (i.e., the D-enantiomer of **4**), also exhibit similar anticancer efficiency in cellular level (Fig. 3A). This relatively comprehensive study of prion-like nanofibers (PriSM) [125], which inhibit cancer cells, illustrates a new direction in the exploration of nanoscale assemblies based on peptides and other related small molecules for potential cancer therapy.

4. *In situ* self-assembly for inhibiting cancer cells

4.1. Enzyme-instructed self-assembly (EISA) of D-peptides for selectively killing cancer cells

Besides improving the efficiency of clinically available drugs and screening potential new drugs, an attractive feature of self-assembled nanostructures is to kill cancer cells selectively. The development of EISA has provided a facile strategy for controlling cell fate according to the enzyme expression level and activity via the *in situ* self-assembly. For example, EISA allows short peptides to enter the cell and to self-assemble to form nanofibers inside the cell for selective inhibition of cancer cells [34]. Moreover, pericellular EISA of D-peptides to form nanofibers can selectively killing cancer cell (Fig. 4A), including drug-resistant cancer cells [127]. For example, ectophosphatases (i.e., the phosphatases on cell surface with active domain outside the membrane) on the cancer cell dephosphorylate a D-tripeptide precursor (Fig. 4B, **6**) to generate self-assembling D-peptide derivative *in situ* (Fig. 4B, **7**), which results in nanofibers/hydrogel in pericellular space of cancer cells. The formed nanofibers cause cell death by blocking intercellular communication and preventing nutrients exchange. The use of Congo red and DAPI to stain nanofibers and cell nucleus, respectively, confirms the pericellular hydrogel formation on a single cell, as the intense red fluorescence around the surface of HeLa cells treated with **6** (Fig. 4C), but hardly shows up on the control cells (i.e., Ect1/E6E7). Besides, DAPI is unable to pass through the cell membrane to nucleus when the cells being treated with **6**, while it can clearly track the nucleus of the control cells. In addition, due to the proteolytic susceptibility of L-peptide, the enantiomer of **6** is unable to show such phenomenon.

The systematic replacement of the L-amino acid in the tripeptide derivative (Fig. 4D, the enantiomer of **6**) by D-amino acid further elucidates the relationship between cell response and conformation of precursors [128]. As shown in Figure 4E, replacement at different residue of **6** results in different cellular responses to the precursors. The precursors that have one or more D-amino acid residues (except enantiomeric pair **12** and **14**) inhibit HeLa cells. These results validate EISA as a process that controls the fate of cancer cell by using enzymatic catalysis to define the spatiotemporal feature of molecular self-assembly. This study also highlighted unusual property of self-assembled D-peptides in biomedical

application. Based on the notion of EISA, Ulijn and Pires used localized biocatalytic self-assembly of an aromatic carbohydrate amphiphile to selectively kill osteosarcoma cancer cells (e.g., Saos-2) [129]. In another relevant study, Maruyama and co-workers used another enzyme (e.g., matrix metalloproteinase-7 (MMP-7)) to modulate the peptide amphiphiles on cell surface for intracellular self-assembly, which results in cancer cell death [130]. These works further validate the use of EISA in extra- or intracellular environment to control the fate of cancer cells [131-133]. Taken the advantages of this system, some other bioactive macromolecules (e.g., antibodies) or small molecules that interact with cell surface receptors could also be combined with EISA to achieve high therapeutic efficiency.

4.2. Intracellular nanostructures formed by D-peptides

With the increasing attention and continuing explorations of D-peptides, one inherent difficulty is the inefficient cellular uptake of D-peptides because nature largely evolves transporters for L-peptides or proteins made of L-amino acids [134, 135]. Current methods for improving the cellular uptake, such as the use of cell penetrating peptides (CPP) or polymeric nanostructure as carrier, remain problematic and inefficient [136-138]. A recent study demonstrates that taurine promotes the cellular uptake of small D-peptides via an enzymatic cleavable linker, which boosts the cellular uptake of the D-peptidic derivative up to 10 folds, and even reaches the concentration of mM [139]. Figure 5A shows the molecular design. Based on this design, after the conjugate of taurine and a D-peptide (**15**) enters cell, the intracellular esterase removes taurine from the ester (**15**), thus the resulted D-peptide (Fig. 5A, **16**) self-assembles. The self-assembled aggregates of **16** decrease the diffusion or efflux of the D-peptide, thus increasing its intracellular concentration (Fig. 5B). Figure 5C, D shows that conjugate **15a**, indeed, acts as a substrate of esterase for EISA. Because of environmental sensitivity of NBD fluorophore, fluorescent microscopy can detect the bright fluorescence resulted from molecular self-assembly [140]. As shown in Figure 5E, strong yellow fluorescence inside HeLa cells treated with **15a** (the substrate having taurine), while there is hardly any fluorescence in HeLa cell treated with **17a** (a control substrate without taurine). Preliminary mechanistic study suggests that the enhanced uptake of **15a**, being independent to taurine transporter, likely involves both dynamin-dependent endocytosis and macropinocytosis. This study illustrates that EISA, in combination with taurine, is an effective strategy for enriching small D-peptides inside cells.

The results in Figure 5 have led to taurine-assisted intracellular EISA of D-peptides to boost the activity of cisplatin against drug-resistant ovarian cancer cells [141]. As illustrated in Figure 6A, esterases (e.g., carboxylesterases (CES)), overexpressed in certain cancer cells, can cleaves taurine from precursor **18**. That is, after **18** enters the cells, intracellular CES catalytically converts **18** to **19**, followed by the self-assembly of **19** to form nanofibers inside the cells. The resulted nanofibers disrupt the formation actin filaments, thus making drug-resistant cancer cells more sensitive to cisplatin. This mechanism is supported by TEM, static light scattering (SLS), cell assay, and cell imaging. TEM reveals the nanofibers in the hydrogels formed by L- and D-enantiomers of **19**, generated by the addition of CES to the solution of L-**18** and D-**18**, respectively (Fig. 6B and C). SLS verifies that the self-assembly of L-**19** or D-**19** occurs at the concentrations that **18** shows significant cytotoxicity to SKOV3 cells. SLS clearly indicates the formation of molecular assemblies at concentration

as low as 10 μ M (Fig. 6D and E). Co-incubation of L-**18** or D-**18** with cisplatin in the cultures of three different ovarian cancer cell lines confirms that L-**18** or D-**18**, while being innocuous to the cells at the optimized concentration, significantly enhance the inhibitory effects of cisplatin towards A2780cis and SKOV3 cells (platinum-resistance ovarian cancer cells) (Fig. 6F and 6G). Fluorescent imaging also reveals that the nanofibers of D-**19** interact with F-actin and interrupt the formation of actin filament (Fig. 6H). These studies illustrate a design of intracellular EISA for potential cancer therapy.

5. Future outlook

Although the introduction of D-amino acids to self-assembling peptides may result in better biostability for the applications of peptidic nanostructures for potential cancer therapy, D-peptides usually has poor specificity to protein targets, which limits their activity and efficacy. A likely solution may come from the development of retro-inverso peptides [142, 143]. But the need of the synthesis of mirror image proteins is rather a challenge. We envision that the development of the virtual screening of retro-inverso peptides may provide a more effective way for generating retro-inverso peptides for self-assembly. Relying on non-covalent interactions, the nanostructures formed by the peptides and drugs usually is less stable *in vivo* than *in vitro*. How to balance these two interactions spatially and temporally is another challenge, as well as an opportunity. We envision the optimal control of the self-assembly and disassembly of nanostructure of the peptides made of D-amino acids ultimately may lead to anticancer processes that not only treat but also prevent cancer. Of course, the development of this kind of process (e.g., reaction-diffusion process, such as EISA, on cell surface) requires better control of nanostructures containing D-amino acids, more accurate characterization of the formed nanostructures [144-147], more precise evaluation of the specific activities of enzyme to those precursors containing D-amino acids, and more extensive study of cancer biology in the presence of D-amino acids.

Additionally, some fundamental issues remain to be resolved: 1) why do the nanostructures of prodrugs usually have lower therapeutic efficiency than parent drugs? 2) The immune responses of self-assembling peptides made of D-amino acids have yet to be evaluated systematically. Although D-peptides exhibit exceptional proteolytic stability, previous studies confirm that the immunogenicity of D-peptides, like L-peptides, largely depend on the sequences of the peptides rather than one the chirality [148, 149]. Considering the assemblies of peptides likely exhibit different properties with those of individual (or monomeric) peptides, it is always necessary to investigate the immunological potential of D-peptides in different states of assemblies, which may lead to pleasant surprises[118, 150] 3) How do the cells respond to the nanostructures made of D-amino acids? And how do the nanostructures interact with the cellular components? 4) Are the nanostructure formed *in vivo* the same as those being observed *in vitro*? 5) Can one rationally design the molecules instead of relying on serendipities? With the advancement of new technologies[144, 151-153], we believe that the development of more adaptive and effective nanostructures based on the peptides made of D-amino acids, ultimately, will provide therapeutic agents to meet these challenges.

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Figure 1.

(A) Molecular structures of Nap-GFFYGRGD (**1**) and Nap-G^DF^DF^DYGRGD (**2**); confocal laser scanning microscopy (CLSM) images of nanofibers formed by **2**. (B) Quantitative biodistribution of ^{125}I -labeled L- and D-peptides in mice after i.v. injection. Tissues were harvested and weighted at 1, 3, 6, and 12 h after initial injection of BALB/c mice, respectively. Data were presented as percent injected dose per gram (%ID/g) $\pm$ standard deviation, $n = 3$. (C) Schemes of L-/D-nanofiber formation and the HCPT encapsulation. (D) L-fiber-HCPT and D-fiber-HCPT inhibited 4T1-luciferase tumor xenograft growth *in vivo*. Tumor inhibition curves and bioluminescent imaging on 4T1-luciferase tumor-bearing mice

20 days after given indicated treatments. Adapted from refs.[82, 94] with the permission from © 2014 American Chemical Society.

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Figure 2.

(A) Binding of the phosphate precursors (**3a** without paclitaxel, presented as CPK model: yellow, phosphorus; red, oxygen.) to the active site of ALP (presented as solid ribbons) and chemical structures of **3a/3b** plus with its optical gel image. (B) TEM images of hydrogel formed by 1.8 wt% of **3a** at pH 7.4 with the catalysis of ALP (1 U/mL) with a scale of 100 nm. (C) IC₅₀ values of paclitaxel, **3a**, and **3b** incubated with HeLa cells after 72 h and (D) relative tumor sizes of mice treated with paclitaxel, L-**3b**, and **3b** for *in vivo* tests. Adapted from ref.[89] with the permission from © 2015 American Chemical Society.

Figure 3.

(A) Molecular structures and dose-responsive curve of **4** (an L-peptide, NapFF) and its enantiomer **5** (a D-peptide) against HeLa cells at 48 h. (B) Negative stained TEM image of the nanofibers formed by **4** ($192 \mu\text{g mL}^{-1}$) in PBS buffer or in in complete culture medium. (C) The mechanism of the selective cytotoxicity of PriSM of **4** toward cancer cell. PriSM of **4** enter the cell by macropinocytosis and impede the cytoskeletal proteins. (D) Inhibitory effect of nanofibers of **4** towards xenograft HeLa tumor on nude mice. (E) Representative image shows mice bearing tumors with similar initial volume (V_0) from each group on 19th day of treatment. White arrows point at tumor. Adapted from refs.[125, 126] with the permission from © 2014, by the American Society for Biochemistry and Molecular Biology and John Wiley and Sons.

Figure 4.

(A) Enzyme-catalyzed formation of pericellular hydrogel/nanonets to induce cell death. (B) Molecular structures of the precursor (**6**) and the hydrogelator (**7**). (C) Overlaid images and 3D stacked z-scan images of Congo red and DAPI stained HeLa and Ect1/E6E7 cell treated by **6** or just culture medium as control for 12 h. HeLa cells treated by **6** at 280 μM ; Ect1/E6E7 cells treated by **6** at 560 μM (scale bar = 10 μm). (D) Molecular structures of the precursors and the corresponding hydrogelators that are enantiomeric isomers. (E) IC_{50} values of different precursors. Adapted from refs.[127, 128] with the permission from John Wiley and Sons and © 2014 American Chemical Society.

Figure 5.

(A) Molecular structures of precursors **15a** and **15b**, and the corresponding hydrogelators **16a** and **16b** after enzymatic transformation. (B) Taurine conjugation boosts cellular uptake of D-peptide precursors and subsequent enzyme-instructed self-assembly to form nanofibers, accumulating inside cells. TEM images of the solutions of **15a** in PBS buffer (C) before and (D) after addition of esterase. Scale bar = 100 nm. (E) Fluorescent confocal microscope images showing the fluorescence emission in HeLa cells with the treatment of (upper) **15a** and (bottom) **17a** at 200 μM concentration in culture medium for 24 h and co-stained with Hoechst 33342 (nuclei) (scale bar = 50 μm). Adapted from ref.[139] with the permission from © 2015 American Chemical Society.

Figure 6.

(A) Enzymatic transformation of the precursor (**18**) as a substrate of carboxylesterase (CES) to the corresponding hydrogelator (**19**) for intracellular self-assembly. TEM images of the hydrogels formed by the addition of CES (2 U/mL) to the solution of (B) L-**18** or (C) D-**18** at the concentration of 0.4 wt% in PBS buffer (Scale bar: 100 nm). The signal intensity ratio of static light scattering (SLS) of the solution of (D) L-**18** or (E) D-**18** at concentrations from 10 to 100 μM before (black bar) and after (gray bar) being treated CES (2 U/mL) for three hours. (F) The cell viability of SKOV3 cells incubated with the precursors D-**18** or L-**18** alone, or in combination with CDDP for 72 h. (G) The cell viability of A2780 cells and A2780cis cells incubated with the precursors D-**18** alone, or in combination with CDDP for 72 h (***=p 0.001, ****=p 0.0001). (H) The fluorescence images of SKOV3 cells stained with Alexa Fluor 633 Phalloidin (F-actin) and Hoechst (nuclei) (upper) after treatment of D-**18** at concentration of 20 μM for 20 h or (bottom) without the treatment of D-**18** (scale bars: left = 20 μm, right = 10 μm). Adapted from ref.[141] with the permission from John Wiley and Sons.