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Biological functions of supramolecular assemblies of small molecules in the cellular environment

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Like biomacromolecules, certain small molecules (*e.g.*, aggregators) are able to self-assemble in the aqueous phase to form nanoscale aggregates. Though it is well-established that the aggregates may interact with enzymes *in vitro*, the study of the biological activities of assemblies of small molecules in the cellular environment is only at its beginning. This review summarizes the recent progress in exploring the biological functions of supramolecular assemblies of small molecules (SASMs). We first discuss the use of SASMs to inhibit pathogenic cells, such as cancer cells and bacteria. The use of SASMs to target different parts of cancer cells, such as the pericellular space, the cytosol, and subcellular organelles, and to combine with other bioactive entities (*e.g.*, proteins and clinically used drugs), is particularly promising for addressing the challenge of acquired multidrug resistance in cancer therapy. Then, we describe the use of SASMs to sustain physiological functions of normal cells, that is, promoting cell proliferation and differentiation for tissue regeneration. After that, we show the use of SASMs as a basic tool to research cell behaviors, for instance, identifying specific cells, improving enzyme probes, revealing membrane dynamics, imaging molecular self-assembly, and mimicking context-dependent signaling. Finally, we give an outlook on the research of SASMs. We expect that this review, by highlighting the biological functions of SASMs, provides a starting point to explore the chemical biology of SASMs.

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1. Introduction

Molecular self-assembly – the spontaneous, noncovalent association of molecules to give higher order structures – is a fundamental cellular process in biology. As a ubiquitous process in nature, endogenous protein monomers self-organize to form supramolecular assemblies (*e.g.*, filaments of actin and tubulin)[1] in the cellular environment, which are indispensable for normal cellular functions (*e.g.*, cell migration, mitosis, and mass transportation). Moreover, it is common for protein assemblies to exhibit emergent properties. For example, the functions of tubulin filaments (*e.g.*, as part of the machinery of mitosis) drastically differ from the functions of a single tubulin (*e.g.*, as a GTPase).[2]

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Like proteins, small molecules also self-assemble to form supramolecular assemblies to exhibit emergent properties that drastically differ from those of the individual molecules. For example, lipid rafts,[3] an endogenous version of assemblies of small molecules (*e.g.*, cholesterols and lipids), remain a notable mystery in cell biology. In fact, advances in several unrelated fields (*e.g.*, biomaterials, drug screening, and neurodegenerative diseases) over the past decade have highlighted the biological importance of supramolecular assemblies of small molecules (SASMs), which are also a form of molecular self-assembly. The development of small molecule hydrogels for biomedical applications such as lanreotide autogel for treating acromegaly[4] clearly validates the application of SASMs in biomedicine. Being able to sequester enzymes or unfolded proteins,[5] block β-amyloid formation,[6] and activate enzymes,[7] SASMs are emerging as a new molecular entity in biomedical research. SASMs are capable of modulating cell differentiation,[8] maintaining cell growth,[9–15] inducing cell death,[7,16–20] inhibiting cancer cell growth *in vivo*,[21] or promoting the proliferation of stem cells.[22] These facts indicate that SASMs are able to interact with cells and to exhibit emergent properties – that is, specific biological functions that are drastically different from their individual molecules[23] – a phenomenon that is more common and important than one previously thought. Thus, it is worthwhile to review the work on the biological functions of SASMs in the cellular environment for the future development of SASMs.

Although considerable work has been published on small molecule assembly with biological applications, such as the applications of liposomes in biomedicine, we choose to review small peptides other than lipids because the structural diversity of peptides offers (almost) unlimited opportunity for designing functions *via* engineering the molecules and the processes of assembly, especially in the context of enzymatic reactions. In the following sections, we first discuss the use of SASMs to inhibit pathogenic cells, such as cancer cells and bacteria, particularly the potential for using SASMs to address the challenges of acquired multidrug resistance in cancer therapy and antimicrobial therapy. Then, we describe the use of SASMs to sustain physiological functions of normal cells, such as promoting cell proliferation and differentiation for tissue engineering. After that, we summarize the use of SASMs as a useful tool to study cell behaviors, for instance, identifying specific cells, improving enzyme probes, revealing membrane dynamics, imaging molecular self-assembly, and mimicking context-dependent signaling. Finally, we discuss the outlook of this emerging research area. We expect this review to provide a starting point to study the chemical biology of SASMs, which is a new direction for exploring the functions of noncovalent synthesis.[24,25]

Bing Xu

After receiving his BS and MS from Nanjing University in 1987 and 1990, Bing Xu obtained his PhD in 1996 from the University of Pennsylvania. Before starting his independent research at the Hong Kong University of Science and Technology in Aug 2000, he was an NIH postdoctoral fellow at Harvard University. He was tenured as an associated professor in Jan 2006 and became a full professor in July 2008 at HKUST. Bing Xu currently is a professor in the Department of Chemistry, Brandeis University. His current research focuses on the applications of enzymatic noncovalent synthesis in materials, biology, and medicine.

2. SASMs inhibit pathogenic cells

Self-assembly of small molecules in water is a rather common observation, as evidenced by the identification of aggregators during drug screening.[7,26,27] High-throughput screening is a common method for identifying drug candidates. Among the filtered products during screening, "false positive" ones that have non-drug-like traits are predominant. This observation stimulates efforts for researching these "futile" substances, consequently finding that the aggregates, not the individual molecules, are the source of the observed biological activities.[28] These results have inspired the study of SASMs, neither the individual constituents[29] nor the precipitates,[30] for various biological functions, such as inhibition of cancer cells or bacteria.[31,32]

2.1 SASMs for killing cancer cells selectively

Cancer is a devastating disease that remains a major threat to public health. The most significant challenge in cancer therapy is adaptive drug resistance caused by genomic instability and the tumor microenvironment even among the initially effective treatments.[33,34] Although cancer would, apparently, have little to do with Alzheimer's disease, an etiological study (*i.e.*, the Framingham Heart Study) on a large population of patients concluded that the patients with Alzheimer's disease have a lower risk of cancers.[35] This serendipitous discovery of the inverse comorbidity between cancer and Alzheimer's disease has a profound implication – that is, supramolecular fibrils formed by self-assembly inhibit cancer cells, and it would be unlikely for cancer cells to develop resistance towards fibrils. This hypothesis has led to selective generation of SASMs *via* enzymatic reactions inside cancer cells for killing the cancer cells. For example, as shown in Fig. 1A, after compound **1a** diffuses into HeLa cells, intracellular esterase catalytically cut off the hydrophilic butyric diacid (**2**) to generate compound **1** inside the cells. The molecules of **1**, having strong intermolecular aromatic–aromatic interactions, self-assemble to form nanofibers, thus increasing the intracellular viscosity and inhibiting HeLa cells.[36] This work also represents the first example of modulating the intracellular phase transition by man-made molecules for controlling the cell fate. A later study shows that SASMs, indeed, minimize acquired drug resistance.[37,38]

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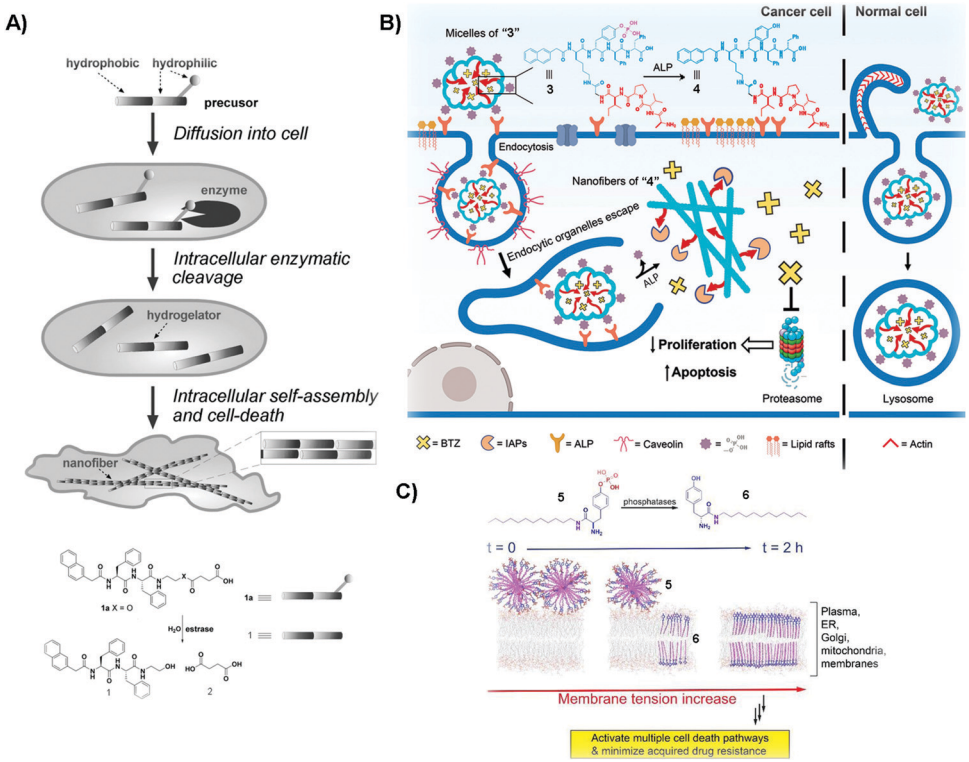

Fig. 1 (A) Illustration of the process of killing cancer cells, and the molecular structures of **1a** and its corresponding products resulting from esterase catalyzed hydrolysis. (B) Molecular structures of **3** and **4** and the diagram of compound **3** inhibiting cancer cells and rescuing normal ones. (C) Molecular structures of **5** and **6** and the illustration of SASMs increasing the membrane tension to inhibit cancer cells. Reproduced with permission from ref. 36, 38 and 40.

One unique property of **1** is that **1** selectively kills cancer cells, but not normal cells. This unique feature promises SAMSs as a fundamentally new molecular entity for developing cancer therapy to increase efficacy, but without increasing toxicity. This notion turns out to be valid and general. As shown in Fig. 1B, compound **3**, a phosphorylated peptide bearing peptide AVPI, an antagonist to the inhibitors of apoptotic proteins (IAPs) that impede the anticancer drug BTZ, is capable of incorporating BTZ. Normally, alkaline phosphatase (ALP), which exists in both the intra- and extracellular space,[39] is overexpressed in/on cancer cells and acts as a cancer biomarker. The quantity of ALP is based on the type of cancer cells.[39] Herein, the micelles of "**3**" interact with ALP from the cell surface to initiate endocytosis to enter cancer cells, and cytosolic overexpressed ALP in these cells cuts off the hydrophilic phosphate groups, converting the micelles of "**3**" to the nanofibers of "**4**", antagonizing IAPs and releasing BTZ to inhibit cancer cells. Herein, ALP, being overexpressed in cancer cells, but not in normal cells, acts as a context-dependent signal. That is, the micelles of "**3**" enter these normal cells by macropinocytosis, and then traffic BTZ into the lysosome because normal expression of ALP is unable to drastically speed up dephosphorylation of the micelles. The trafficking of BTZ to lysosomes thus minimizes the side effects of BTZ on normal cells.[40] In this study, the enzymatic reaction enables selectively generating functional SASMs in cancer cells, but not in normal cells. This approach should be applicable to the use of other enzymes to form SASMs for targeting cancer cells.

Multidrug resistance (MDR), which emerges in patients receiving long-term anticancer medication, is a major challenge in cancer therapy. In the complex tumor microenvironment, lipids are able to control multidrug-efflux pumps in cancer cells, and their dysfunction leads to MDR in cancer.[41] Therefore, lipids represent an important and challenge target for countering MDR because of the large quantity and heterogeneity of lipids.[42] Designing SASMs to target lipid membranes promises an effective approach against MDR cells. As shown in Fig. 1C, a lipid derivative (**5**) self-assembles into micelles. ALP, overexpressed on cancer cells, catalyzes the dephosphorylation of **5** to insert the self-assembled **6** into membranes. This process increases the tension of the cell membrane, thus stimulating a variety of regulated cell-death pathways and minimizing acquiring MDR.[38] In the above studies, the SASMs exhibit emergent properties because individual molecules (*i.e.*, without assembling) are innocuous to cells. Moreover, SASMs, being generated by a specific enzymatic reaction, are also able to interact with multiple targets or to disrupt multiple pathways in cancer cells,[43] which makes SASMs attractive molecular entities for killing cancer cells effectively, reducing side effects, and minimizing MDR in cancer therapy.[44,45]

2.1.1 SASMs form at pericellular and tandem locations. Besides intracellular targeting as mentioned above, the pericellular space is another location for producing SASMs to efficiently inhibit the growth of MDR cells. As shown in Fig. 2A, overexpressed ALP, as an ectoenzyme on cancer cells (*e.g.*, HeLa cells), catalytically removes the phosphate group from the D-peptide derivative (**7**).

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This process results in the formation of a pericellular hydrogel of SASMs, hindering the exchange between intra- and extracellular mass, triggering apoptosis of several kinds of cancer cells, especially MES-SA/Dx5, an MDR cell phenotype.[46] Besides ALP, other bioactive molecules overexpressed by surrounding cancer cells, such as cell membrane receptors, can facilitate the formation of SASM-based pericellular hydrogels.[47]

For certain cancer cells, targeting only one extra- or intracellular location is insufficient to effectively inhibit cancer cells. Thus, tandem molecular self-assembly, acting as an approach to increase the sophistication of SASMs, is able to interact with cancer cells more extensively at multiple locations for increasing the anticancer efficacy. As shown in Fig. 2B, ALP, acting as a key factor, hydrolyzes **9** to remove the phosphate group from **9**. The resulting molecule, **10**, self-assembles to form nanoparticles, which adhere to cancer cells because of the RGD motif, a ligand of integrin, in **10**. When the assemblies of **10** enter cells and are reduced by glutathione (GSH), the nanoparticles lose the hydrophilic part (ERGD) to generate nanofibers made of **11**. Being superior to the self-assembly controlled only by ALP, this tandem molecular assembly is able to inhibit cancer cells efficiently. Because cancer cells express cancer biomarkers, such as ALP and GSH, at higher levels than normal cells do, ALP and GSH contribute to the high efficacy of **9** against cancer cells.[39] This approach also provides a way to target cancer cells with more precise spatial control. The principle of tandem molecular assembly should be useful for targeting multiple other targets or locations, such as subcellular organelles, for countering MDR and reducing adverse effects in cancer therapy.

2.1.2 SASMs target subcellular organelles. Because mitochondria play a central role in many cellular processes,[48–54] SASMs targeting the mitochondria of cancer cells are another way to increase the anticancer effect and reduce drug resistance. As shown in Fig. 3A, upon ALP catalyzed hydrolysis, **12**, a peptide derivative bearing mitochondria-targeting triphenylphosphonium (TPP),[55] turns into **13**, which self-assembles on the cancer cells. After entering the cancer cells, the assemblies of **13** target and disrupt the mitochondria to activate the intrinsic cell death pathway, and then effectively inhibit the cancer cells (*i.e.* Saos-2 cells) without acquired drug resistance.[56]

An unexpected molecule for targeting mitochondria is **14**, a substrate of enterokinase (ENTK). ENTK, in fact, is a protease, which presents on the mitochondria of HeLa cells.[57] ENTK-instructed self-assembly generates SASMs for targeting mitochondria of cancer cells. As shown in Fig. 3B, **14**, the peptide derivative bearing a Flag tag (*i.e.* a protein tag),[58] forms micelles. In HeLa cells, the cleavage of the Flag by ENTK turns the micelles to nanofibers, which are largely located on mitochondria. The micelles formed by the precursor (**14**) are capable of delivering doxorubicin (Dox), an anticancer drug, into the cancer cells, and then the Dox is mainly released to mitochondria, which causes increased cytotoxicity against HeLa cells compared to Dox only.[57] This is the first report to employ enzymatic noncovalent synthesis[24] for targeting mitochondria, complementing the "TPP" approach.[56] This approach should be applicable to the use of other enzymes and to inhibit other cancer cells, which also stimulates efforts to design more precursors that allow enzymatic reactions to generate SASMs at mitochondria of cancer cells, as shown by the recent work of Sun *et al.*[59] In addition, the process of forming SASMs catalyzed by ENTK also finds applications for delivering genes selectively into mitochondria of cancer cells.[60]

The process of generating SASMs by ENTK is also able to traffic proteins, such as histone 2B, to mitochondria. H2B normally localizes in the nucleus and is absent in mitochondria. Although a proteomic analysis reports the existence of H2B in the mitochondria of a liver cancer cell,[61] the detailed mechanism remains to be elucidated. Interestingly, ENTK catalyzed SASMs traffic H2B into mitochondria, which may provide a way for better understanding inter-organelle communication in cancer cells. As shown in Fig. 3C, **15** is a molecule similar to **14**, both of them having the Flag tag. The molecule of **15** is able to interact

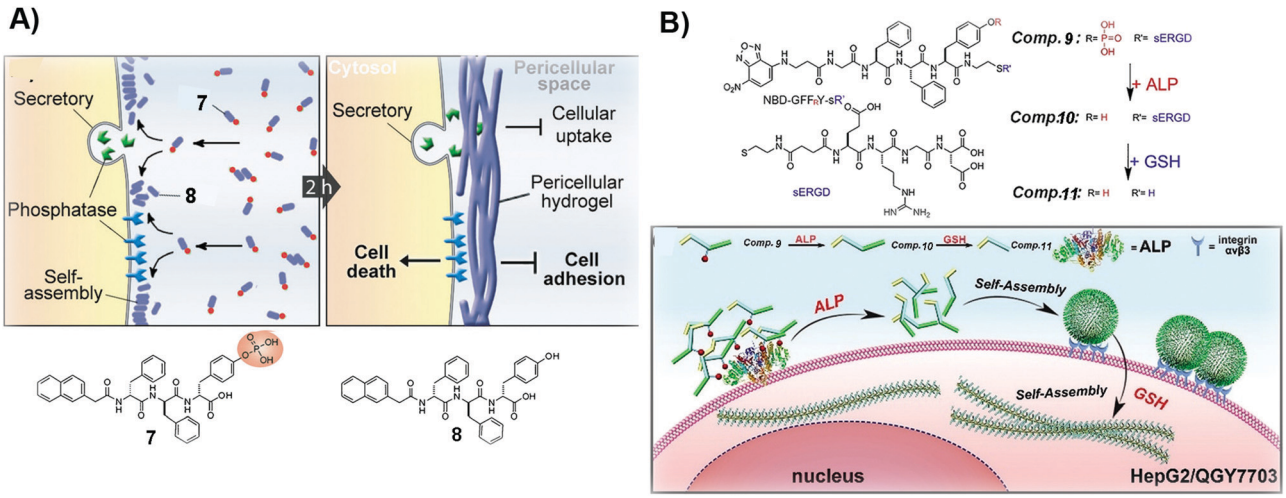

Fig. 2 (A) Illustration of the formation of a pericellular hydrogel and the molecular structures of **7** and **8**. (B) Molecular structures of **9–11** and the diagram of the process of tandem self-assembly. Reproduced with permission from ref. 39 and 46.

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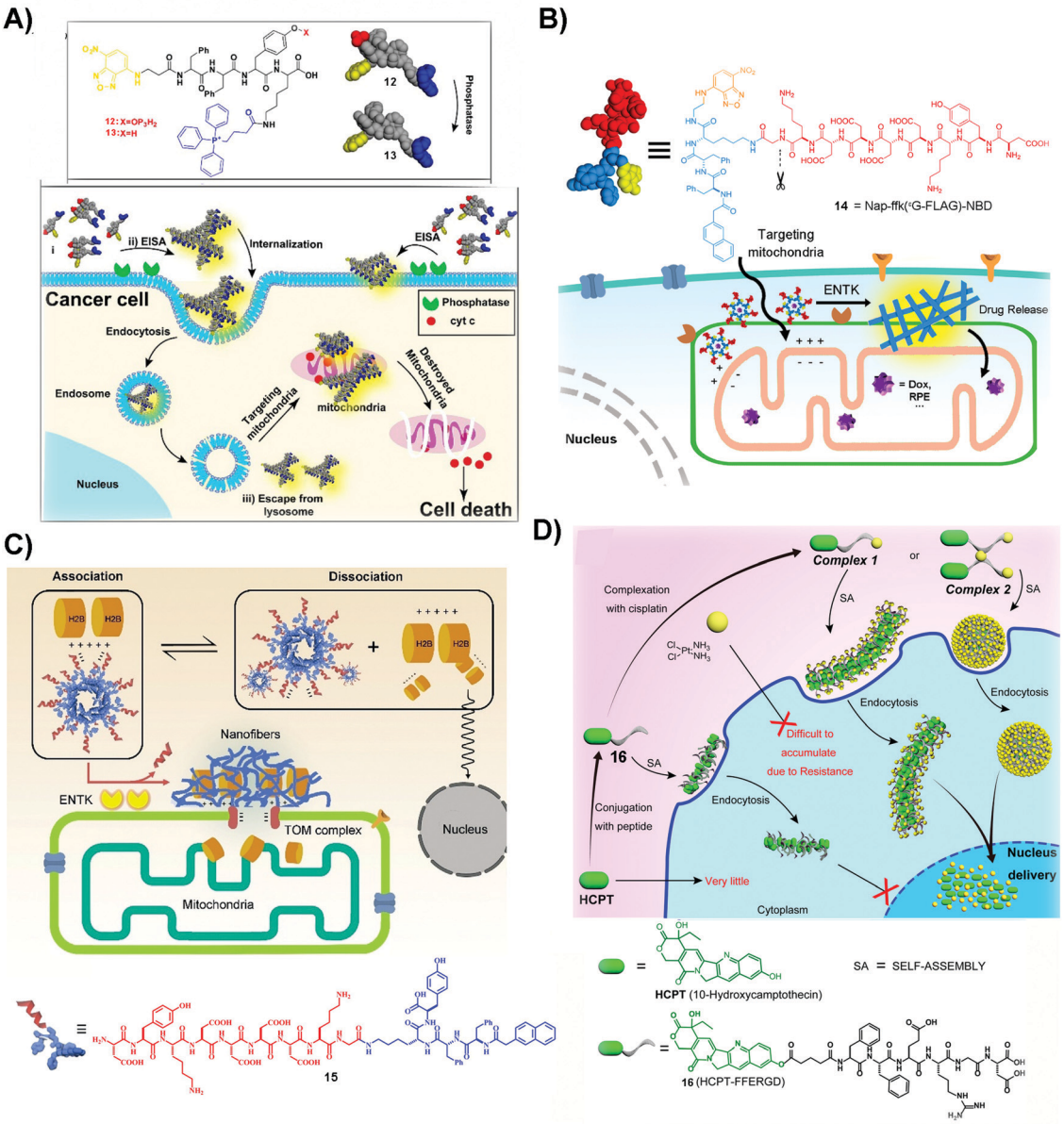

Fig. 3 (A) The structures of **12** and **13** and the illustration of **12** inhibiting cancer cells. Reproduced with permission from ref. 56. Copyright 2016, American Chemical Society. (B) The structure of **14** and the diagram of **14** targeting mitochondria by ENTK-instructed self-assembly. Adapted with permission from ref. 57. Copyright 2018, American Chemical Society. (C) Illustration of retention of H2B on mitochondria and the molecular structure of **15**. Reproduced with permission from ref. 62. (D) Illustration of a dual drug system to locate the nanofibers and nanoparticles at the nucleus and the molecular structure of **16**. Adapted with permission from ref. 64. Copyright 2017, American Chemical Society.

with H2B, preventing H2B into nucleus. With the catalysis of mitochondrial ENTK, **15** loses the Flag tag and self-assembles to form nanofibers, which retain H2B and traffic this protein into mitochondria. In addition, the cytotoxicity of a combination of **15** and Dox to cancer cells is higher than that of Dox alone.[62] This method underscores the use of SASMs for manipulating inter-organelle crosstalk, which remains underexplored. In addition, this method should be applicable to combine other clinical drugs with SASMs for inhibiting cancer cells effectively.

The past few years have seen the exploration of SASMs for targeting different organelles in cancer therapy. Besides mitochondria, SASMs are also able to target other organelles, such as the endoplasmic reticulum[63] and nucleus.[64,65] One of the merits is that SASMs can deliver clinical anticancer drugs, which interact with DNA or DNA-associated proteins, into the nucleus. As shown by the work of Yang *et al.*, SASMs traffic cisplatin and 10-hydroxycamptothecine (HCPT) into the nucleus to increase the anticancer efficacy. In that study, cisplatin, by chelation of itself with a carboxylate of an amino acid, is able to bond with **16** to form a dual drug-delivery system (Fig. 3D). Adding 1 and 1.5 equivalents of cisplatin into compound **16** forms complex 1 and 2, which self-assemble to form nanofibers and nanoparticles, respectively. These nanofibers or nanoparticles are able to accumulate in the nucleus, inhibiting

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cancer cells effectively because cisplatin chelates with DNA to inhibit DNA synthesis and HCPT antagonizes DNA topoisomerase I.[64,66,67] Another dual drug design, in which the chelated cisplatin is up to 8 equivalents, is able to achieve superior nucleus accumulation when the actual loading of cisplatin is 4 equivalents. This approach offers excellent inhibitory efficiency against cancer cells,[65] thus promising the use of SASMs to target subcellular organelles for high inhibitory activity against cancer cells and to engineer more molecules to form higher-order assemblies for countering MDR in cancer therapy.

2.1.3 SASMs combine with other bioactive entities. In certain cases, SASMs have to reach relatively high concentrations to kill cancer cells. Thus, it is reasonable to combine SASMs with other bioactive constituents, such as proteins, clinically used anticancer drugs, antibiotics, and photosensitizers, as a versatile approach to synergistically inhibit cancer cells.[68–72] For example, because human epidermal growth factor receptor 2 (HER2), being overexpressed in some breast and gastric cancers, is a validated therapeutic target,[73] combining SASMs and an antibody against HER2 (anti-HER2) indeed acts as an efficient means to inhibit the

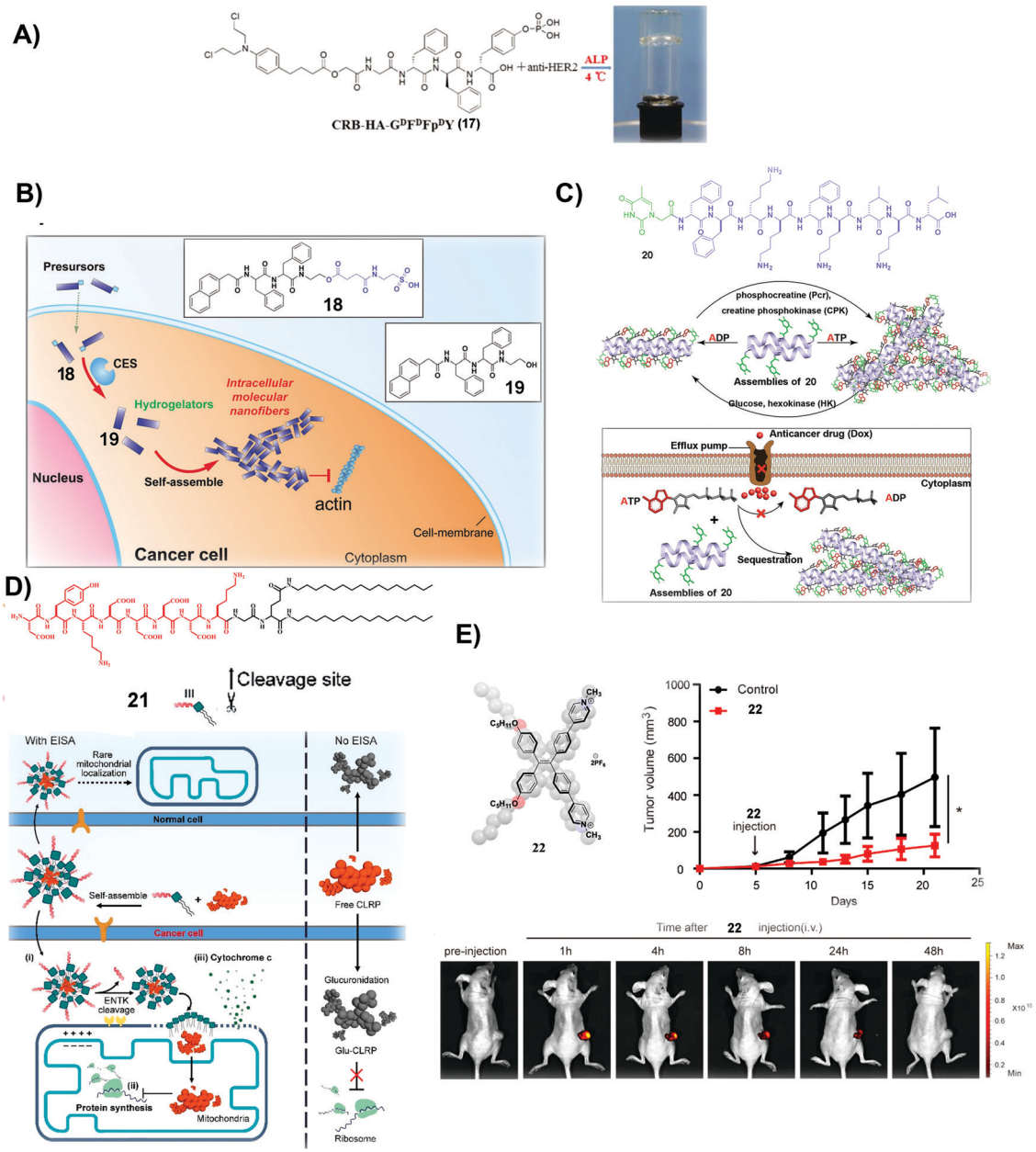

Fig. 4 (A) Molecular structure of **17** and optical image of the co-assembled hydrogel. (B) Molecular structures of **18** and **19** and illustration of the process of self-assembly. (C) Molecular structure of **20** and the illustration of the nanostructure change by interconverting ATP and ADP and the mechanism of slowing down the efflux pumps to retain DOX. (A–C) Reproduced with permission from ref. 68–70. (D) Molecular structure of **21** and the plausible mechanism of **21** and CLRP inhibiting cancer cells over normal ones. (E) Molecular structure of **22** and measurement of tumor volume and fluorescent visualization of tumors after intravenous injection of **22** in tumor-bearing mice. (D and E) Adapted with permission from ref. 71 and 72. Copyright 2020, American Chemical Society.

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proliferation of HER2-positive cancer cells. As shown in Fig. 4A, chloroambucil (CRB) is a clinical anticancer drug. A CRB-capped peptide (**17**) encapsulates anti-HER2; under ALP-catalyzed hydrolysis, **17** loses the phosphate group, and the resulting molecule assembles with anti-HER2 to form a hydrogel. This co-assembled hydrogel specifically binds to HER2-positive cancer cells (*i.e.* NCI-U87 cells), and exhibits superior anticancer activity to CRB alone.[68] These findings demonstrate the excellent performance of SASMs, which promises a facile, yet fundamentally new way to target cancer cells. This work also provides a useful guiding principle to use other combinations of SASMs and antibodies for inhibiting other cancer cells.[74]

Besides covalently conjugating a clinically used anticancer drug (*e.g.*, CRB) to small molecules to form SASMs (*e.g.*, SASMs of **17**), co-incubating first-line anticancer drugs and SASMs provides a simpler method to inhibit cancer cells.[69,70] Fig. 4B shows an amphiphile molecule **18**, which is a substrate of carboxylesterase (CES), an enzyme overexpressed in ovarian cancer cells. After entering cells and being hydrolyzed by CES, **18** turns into **19**, which self-assembles to form nanofibers. Co-incubation of **18** and cisplatin boosts the activity of cisplatin against SKOV3 and A2780cis cells, two kinds of drug-resistant ovarian cells.[69] This method, based on SASMs, considerably increases the anticancer efficacy and reduces MDR. Thus, SASMs should be applicable to sensitize cancer cells to other first-line anticancer drugs for enhancing the efficacy of anticancer drugs when resistance to those anticancer drugs emerges.

Because efflux of anticancer drugs is a common mechanism for MDR in cancer therapy, targeting the efflux pumps is a promising approach against MDR. As shown in Fig. 4C, a nucleopeptide, **20**, of which the capping group is thymine, is able to form SASMs for sequestering ATP. Because efflux pumps rely on ATP to be functional, sequestering ATP by SASMs of **20** impedes the efflux of drugs, thus reducing MDR. For example, co-incubation of **20** and doxorubicin (DOX), an anticancer drug, is capable of retaining DOX to inhibit MDR cancer cells.[70] This work highlights the use of SASMs to impede MDR in cancer therapy, which contributes to the efforts to search for more SASMs to encapsulate clinical drugs for increasing the toxicity of these drugs against MDR cancer cells.

Combining antibiotics with SASMs also drastically increases the inhibitory efficacy of the antibiotics against cancer cells. Chloramphenicol (CLRP), a clinically approved antibiotic, is ineffective to inhibit live cancer cells because cytosolic glucuronidase detoxifies it.[75] A peptide–lipid conjugate, **21**, as a substrate of ENTK on mitochondria, forms micelles to encapsulate CLRP. Being catalytically hydrolyzed by ENTK to lose the Flag, the micelles become SASMs and release CLRP into mitochondria of cancer cells, as shown in Fig. 4D. Because mitochondria lack antagonistic glucuronidases to detoxify CLRP, CLRP inhibits mitochondrial protein synthesis and interrupts the metabolism of these liver cancer cells. Such inhibition of mitochondrial protein synthesis sensitizes the cancer cells to cisplatin.[71] This intriguing phenomenon expands the applications of SASMs to repurpose clinically validated drugs for effectively suppressing cancer cells and reducing the systemic burden.

Photodynamic therapy (PDT) is a useful approach to minimize MDR. Therefore, a photosensitizer is another kind of molecule for combining with SASMs to inhibit cancer cells more efficiently. Based on the design principle of theranostics, which integrates diagnosis and therapy into the same molecular platform, fluorescent molecule assemblies are another type of SASMs for cancer theranostics. As shown in Fig. 4E, **22**, which generates fluorophore SASMs formed by the aggregation of a fluorophore, is able to act as a PDT agent for imaging and inhibiting colon tumors simultaneously.[72] This study expands the molecular space of SASMs to fluorescent and phototoxic small molecules that aggregate in aqueous phases.

2.2 SASMs for inhibiting bacteria specifically

Microbial infection lowers the quality of life. In some acute and severe microbial infections, such as in osteomyelitis, amputation or death usually happens. Despite the development of antibiotics for reducing the mortality and morbidity of patients, the emergence of antimicrobial drug resistance remains a threat to public health. Generally, effective targeting and increasing treatment efficacy are two main approaches to counter MDR.[76,77] The unique merits of SASMs, such as acting over multiple length scales and quick responses to biological cues,[78–80] make them attractive candidates for overcoming MDR. For example, lipid-based SASMs are capable of switching their anti-infectious ability "on" and "off" in response to changes of pH.[81] Moreover, some enzymes, being overexpressed on or in microbial cells, are able to act as a trigger to form SASMs for specifically inhibiting bacteria.[82] In addition, combination therapy, such as using antibiotics or proteins to combine with SASMs, should inhibit microbial invasion more efficiently than each single agent. In this section, we mainly discuss enzymes as the trigger for inhibiting microbial infection and combining SASMs with other bioactive entities, such as antibiotics and proteins, to inhibit infection and to reduce MDR.

2.2.1 Enzymes as the trigger to inhibit bacteria. Esterase-catalyzed SASMs are able to improve the therapy efficacy against Gram-negative bacteria and lower toxicity to normal cells. As shown in Fig. 5A, chloramphenicol succinate (**24**) conjugated with diglycine (GG) generates a prodrug, **25**. In *E. coli*, **25** undergoes fast catalysis by intracellular overexpressed esterase and loses succinate-GG to form **23**, which self-assembles to form nanoparticles to inhibit the bacteria. More importantly, compared to **23**, **25** has lower toxicity to bone marrow stromal cells, which would reduce the systemic burden.[83] This method likely would be applicable to target other enzymes, such as matrix metalloproteinases[84] and gelatinase,[85] for minimizing MDR.

Broad-spectrum antibiotics, such as chloramphenicol (**23**), have the merit of killing many varieties of microorganism, but they are more likely to result in MDR, especially in that patients usually undergo long term administration of this type of antibiotics. Using SASMs to target gelatinase is able to specifically inhibit one certain kind of bacteria over another one. As shown in Fig. 5B, the fusion peptide (**26** or **27**) consists of an antimicrobial peptide (red part), a recognition sequence (blue parts) that is the substrate of bacterial gelatinase,[86] and a cellular adhesion

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sequence (green part). As shown in Fig. 5C and D, **26** and **27** are able to generate 2D assemblies on a poly(methyl methacrylate) (PMMA) surface, which exert inhibitory activity against bacteria by gelatinase-catalyzed cleavage of the assemblies of **26** and **27**. While the assemblies of **26** kill both *S. aureus* and *E. coli*, the assemblies of **27** are able to differentiate *S. aureus* and *E. coli* strains,[85] likely due to the difference in the bacterial cell membranes. This study provides an approach to design SASMs to kill a certain kind of microbial cells over another one, which may pave the way to design SASMs as narrow-spectrum antibiotics for reducing the odds of evolving antimicrobial MDR.

2.2.2 SASMs combine with antibiotics and proteins. Vancomycin, a kind of antibiotic, is able to bind D-Ala-D-Ala of Gram-positive bacterial cell walls to inhibit the growth of the bacteria. But vancomycin-resistant bacteria convert D-Ala-D-Ala to D-Ala-D-lactate, reducing the affinity of the bacterial cell wall to vancomycin and leading to drug resistance. Assemblies of vancomycin derivatives are able to address this issue. As shown in Fig. 6A, vancomycin derivatives like **28** and **29** are capable of self-assembling on the surface of Gram-positive bacteria, inhibiting the bacteria, especially drug-resistant ones (see Fig. 6B).[87] This work should be applicable to combine SASMs with other antibiotics, such as triclosan,[88] for enhancing the antibacterial capability.

Photodynamic inactivation (PDI) is to damage the bacterial membrane and induce bacterial death by photoirradiation. The advantage of PDI is that it minimizes the odds of MDR. But obtaining photosensitizers that have the ability to intercalate bacterial cell membranes is challenging. Combining SASMs that incorporate the photosensitizer with a protein leads to the intercalation of cell membranes and enhanced PDI efficacy against bacteria. As shown in Fig. 6C, the photosensitizer, **30**, and the transacting activator of transduction (TAT) peptide-decorated protein (**31**), which has the ability to insert into the membranes, self-assemble to form nanoparticles, which intercalate cell membranes, promote ROS production, and boost the PDI efficiency to inhibit bacteria.[89] This study underscores the combination of SASMs with proteins to inhibit bacteria, which may stimulate the design of other SASMs to combine with proteins against MDR without the need for light.

3. SASMs for maintaining physiological functions of normal cells

SASMs find applications not only in eliminating pathogenic cells, but also in sustaining physiological functions of normal cells, such as promoting cell proliferation and differentiation for tissue engineering, as discussed in the following section.

3.1 SASMs promote cell proliferation and differentiation

SASMs are able to mimic the extracellular matrix (ECM) for maintaining cell proliferation. As shown in Fig. 7A, **32**, being reduced by TCEP, forms **33**, which self-assembles to form a hydrogel. The hydrogel undergoes directional domain sliding motion by the hydrophobic collapse transition. The sliding motion within the hydrogel accelerates cell proliferation by mechanotransduction, which translocates yes-associated protein, YAP, from the cytosol into the nucleus.[90] This work highlights SASMs to mimic the ECM to promote cell proliferation, which implies the promise of SASMs in other fields, such as in cell differentiation.

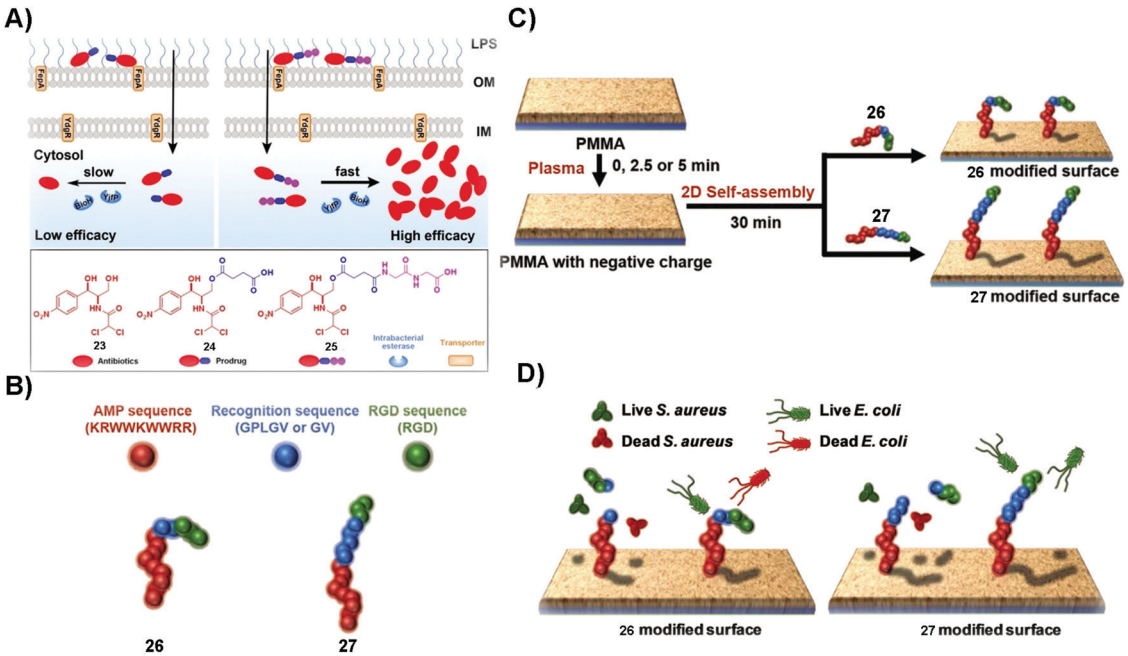

Fig. 5 (A) Plausible mechanism of **25** to inhibit Gram-negative bacteria more effectively than **24** and the molecular structures of **23**, **24** and **25**. (B) The construction of the fusion peptides, **26** and **27**. (C) The generation of fusion peptide (**26** or **27**)-modified surfaces. (D) The assemblies of **26** kill both *S. aureus* and *E. coli* and those of **27** distinguish *S. aureus* and *E. coli* strains and kill the bacteria selectively. Reproduced with permission from ref. 83 and 85.

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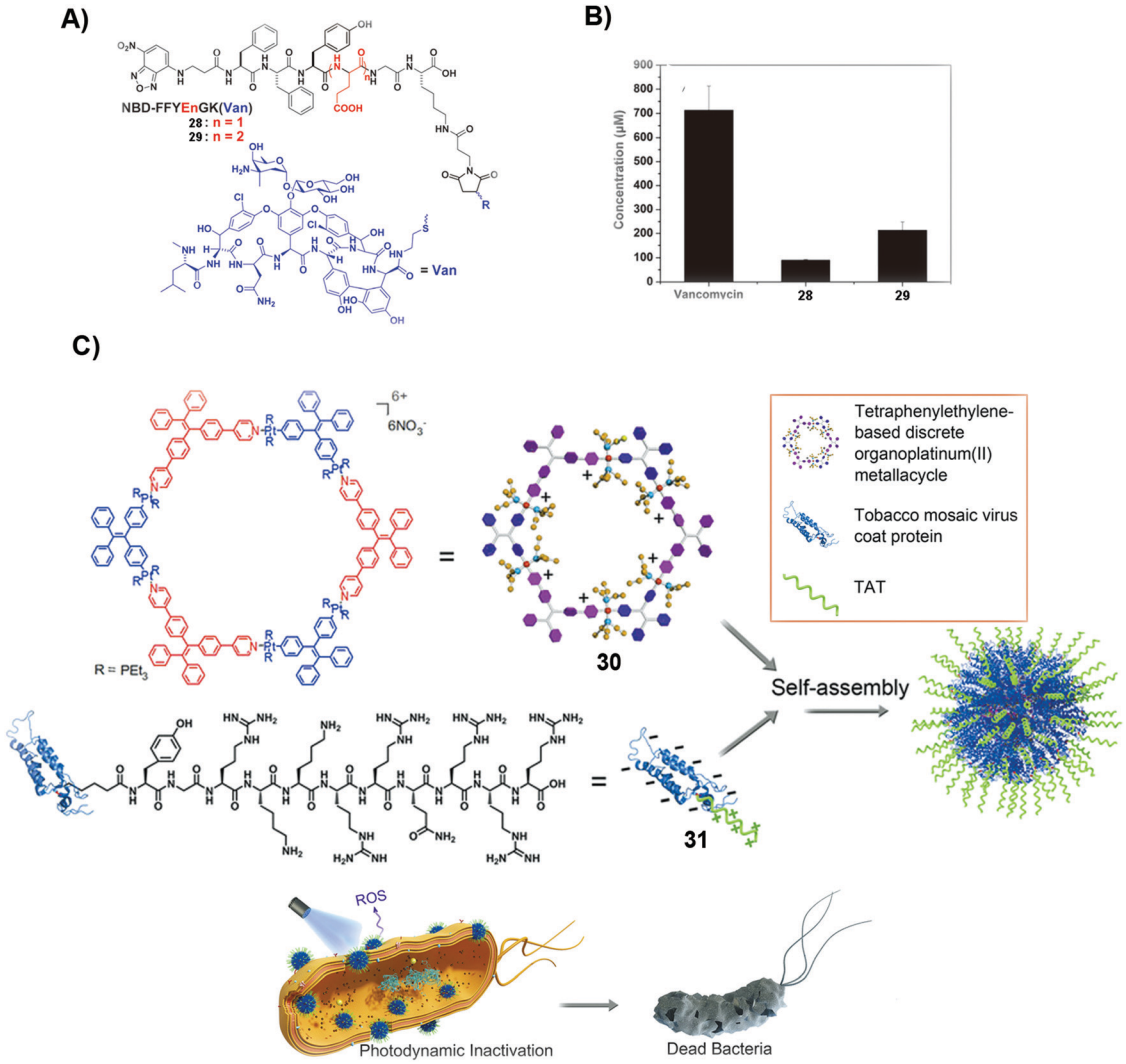

Fig. 6 (A) Molecular structures of **28** and **29**. (B) The minimum concentrations of different compounds, such as vancomycin, **28** and **29**, to inhibit drug-resistant bacteria, *E. faecalis*. (A and B) Reproduced with permission from ref. 87. Copyright 2014, Royal Society of Chemistry. (C) Mechanism of deactivating and inhibiting bacteria by SASMs combined with proteins. Reproduced with permission from ref. 89.

Traumatic injuries in the central nervous system (CNS) result from damaging nervous tissue. Thus, it is attractive to use hydrogels made of SASMs to mimic the ECM for the regeneration of neurons. Since laminin, as an indispensable protein of the ECM, plays a critical role in cell differentiation during nerve regeneration,[91] SASMs, as a mimic of laminin in the ECM, are able to bias the differentiation of neuron progenitor cells for forming neurites, which promises a potential approach for repairing the CNS. As shown in Fig. 7B, **34**, based on the laminin-mimetic peptide IKVAV, self-assembles to form nanofibers, which drive differentiation of human bone marrow mesenchymal stem cells (BMSCs) to the neural lineage.[92] Besides nerve cells, mesenchymal stem cells (MSCs) are able to differentiate into other cells as therapeutics in tissue engineering. For example, SASMs are able to prompt bone and cartilage generation, which provides a guideline for discovering metabolites that may be relevant to therapeutic drugs.[93] These studies illuminate a promising direction for SASMs in cell proliferation and differentiation, which, ultimately, may make applications of SASMs in tissue engineering possible.

3.2 SASMs for ischemic hind-limb salvage, sarcopenia treatment, and acute kidney injury (AKI) repair

Insulin-like growth factor-I (IGF-1) plays important regulatory functions in tissue engineering.[94] GYGSSSRRAPQT, the C region of IGF-1 (named IGF-1C), which interacts specifically with the IGF-1 receptor (IGF-1R),[95] emerges as a functional mimic of IGF-1. IGF-1C, however, exhibits an unstructured conformation by itself, making it less effective to activate IGF-1R.[96] SASMs of IGF-1C, adopting a β-sheet conformation to act as a mimic of IGF-1, not only bind with IGF-1R, but also activate it, evidenced in the study of ischemic hind-limb salvage. As shown in Fig. 8A, **35**, of which the green part is IGF-1C, self-assembles to form assemblies that adopt a β-sheet conformation. The experimental result shows that the β-sheet-conformational IGF-1C, in the form of a hydrogel, has strong affinity to IGF-1R.

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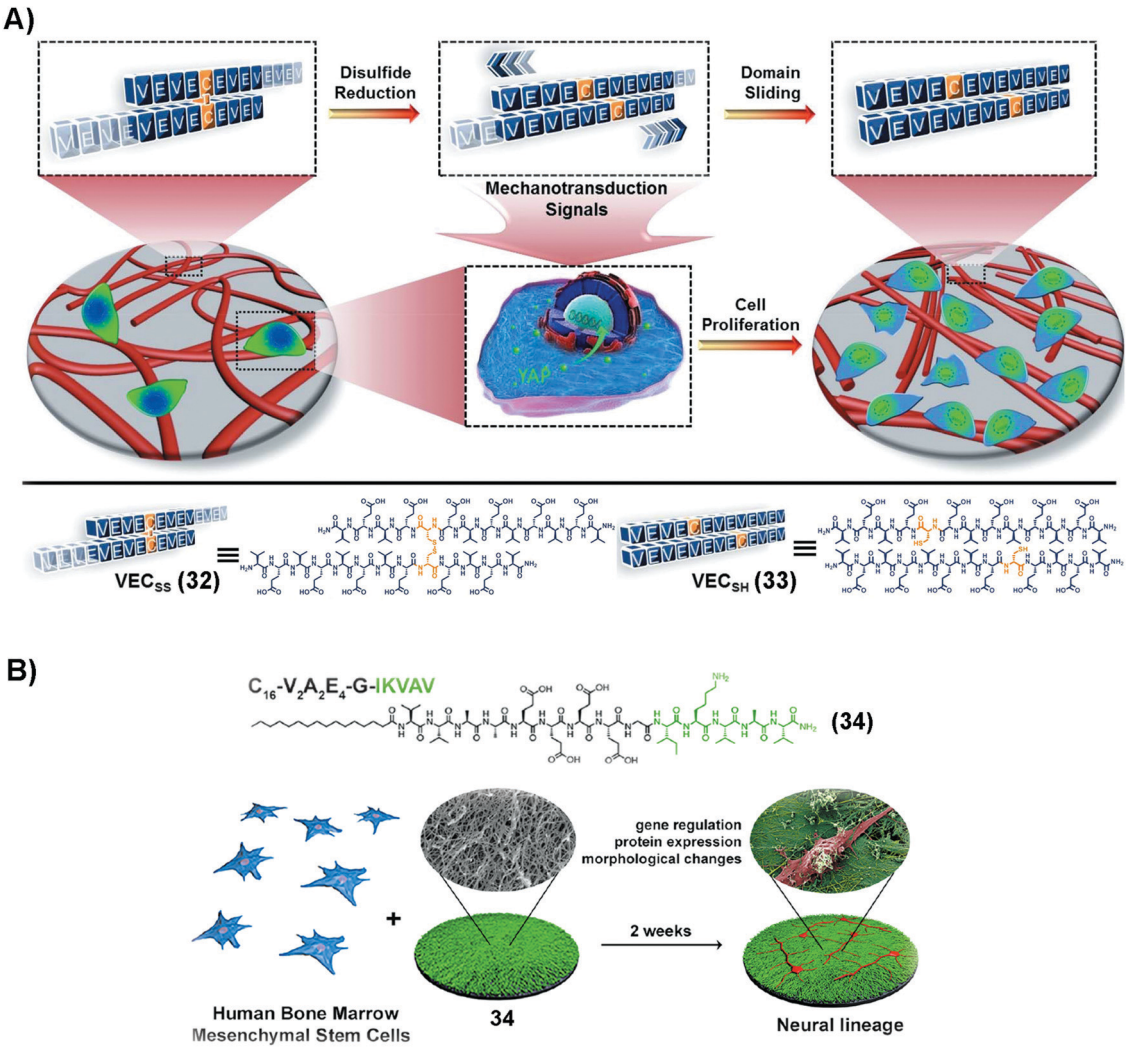

Fig. 7 (A) Molecular structures of **32** and **33** and the process of formation of the hydrogel and possible mechanism of the hyrdogel stimulating cell proliferation. Adapted with permission from ref. 90. Copyright 2020, Royal Society of Chemistry. (B) Molecular structure of **34** and illustration of neural differentiation by adding BMSCs on **34**, which self-assembles into nanofibers. Adapted with permission from ref. 92. Copyright 2018, American Chemical Society.

Moreover, it exerts superior ability to enhance ischemic hind-limb salvage than the IGF-1 protein, by activating the downstream signaling pathway.[97] Encouraged by this study, other SASMs, which also mimic IGF-1, are capable of treating other diseases, such as treating sarcopenia and AKI.[98,99] As shown in Fig. 8B, an ultrashort sequence SSSR substitutes IGF-1C in **35**, forming compound **37**. Fig. 8C and D show that assemblies of **37** reduce the myoblast damage in a dexamethasone (Dex)-instructed sarcopenia model.[98] Additionally, as shown in Fig. 8E, **38**, an amphiphile molecule similar to **35** but having D-formed amino acids, self-assembles to form a hydrogel. The hydrogel improves the activity of human placenta-derived mesenchymal stem cells (hP-MSCs) to rescue murine in an AKI model.[99] Besides acting as a carrier medium of MSCs, SASMs are also able to incorporate other bioactive entities, such as excellular vesicles, for increasing the therapeutic effiency of the excellular vesicles in AKI.[100] These studies highlight the promise of SASMs for application in tissue engineering.[101]

4. SASMs as a tool to study cell behaviors

SASMs are not only capable of inhibiting pathogenic cells and maintaining physiological functions of normal cells, but also are able to act as a useful tool to study cell behaviors, such as revealing protein–peptide interactions. As shown in Fig. 9, 4,7-di(thiophen-2-yl)-2,1,3-benzothiadiazole (DBT), a fluorescent molecule, conjugated to two molecules of a peptide (EEGWRESAI) that binds with tax-interacting protein-1 (TIP-1) generates molecule **39**. Because of the environmentally sensitive fluorescence of DBT, **39** interacts with TIP-1 or a tetramer protein, ubiquitin-like domain protein (ULD) and TIP-1 (ULD-TIP1), to exhibit far-red/near-infrared (FR/NIR) fluorescence, after forming spherical nanoaggregates or nanofibers. Besides, in solution, this interconverting fluorescent switch of "on" and "off" with high contrast is able to report peptide–protein interactions in bacteria.[102] In addition to studying peptide–protein interactions,

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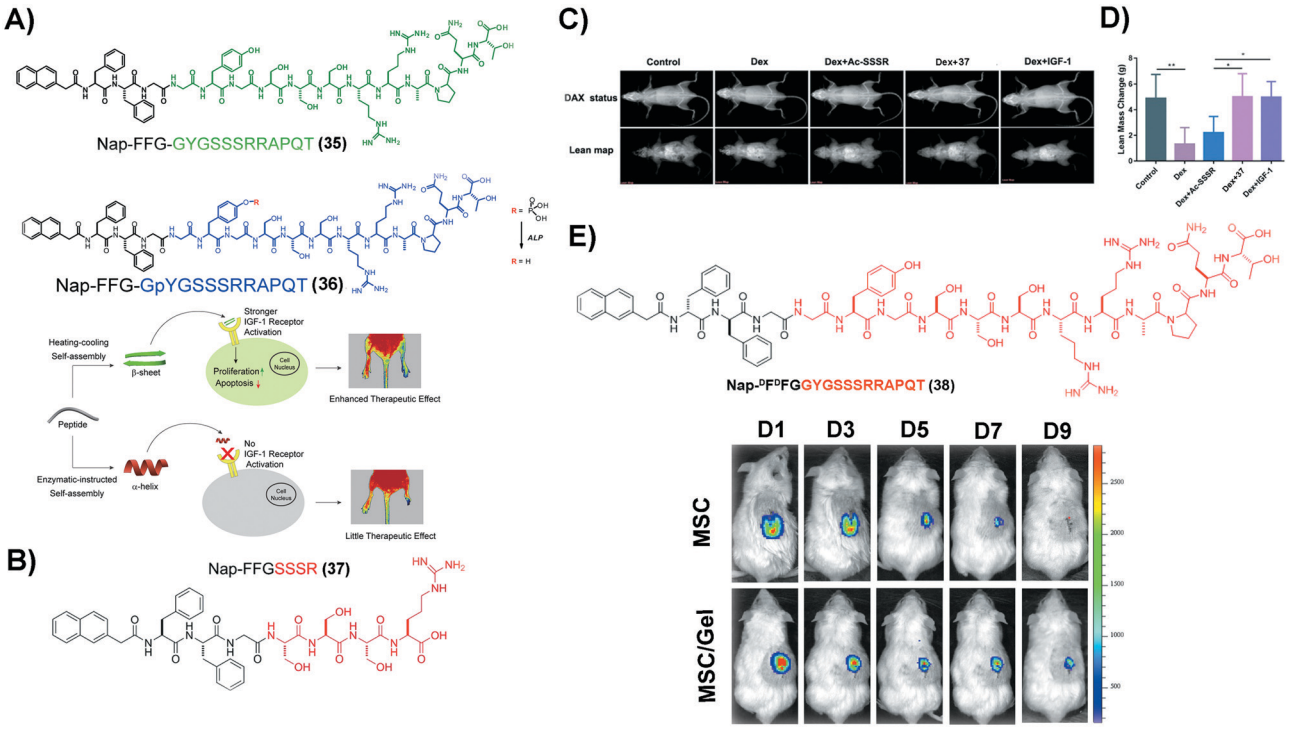

Fig. 8 (A) Molecular structures of **35** and **36** and illustration of the corresponding hydrogels reparing the ischemic hind-limb. (B) Molecular structure of **37**. (C) Lean mass changes after different treatment, using dual-energy X-ray absorptiometry (DXA). (D) Caculation of the lean mass in (C). (A–D) Adapted with permission from ref. 97 and 98. Copyright 2019 and 2020, American Chemical Society. (E) Molecular structure of **38** and tracking of hP-MSCs, which are labelled by luciferin activities, in an AKI model over a period of 9 days. Reproduced with permission from ref. 99. Copyright 2020, Dove Medical Press.

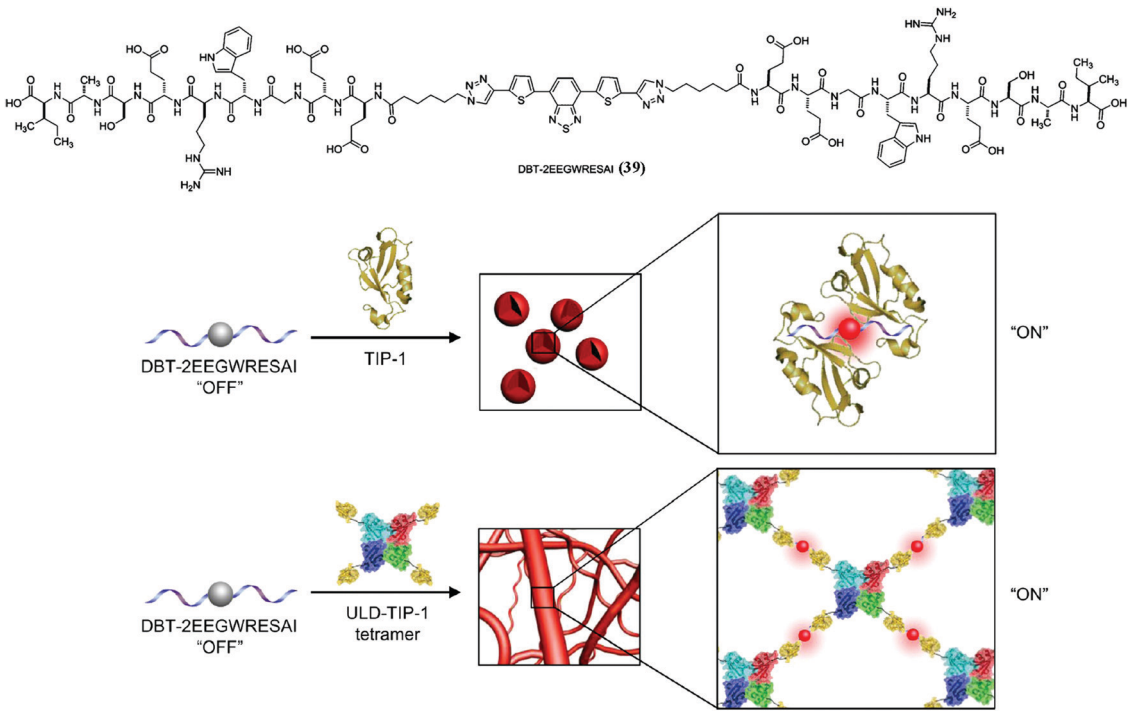

Fig. 9 Molecular structure of **39** and illustration of lighting up the fluorescence of **39** to monitor peptide–protein interactions. Adapted with permission from ref. 102. Copyright 2014, American Chemical Society.

SASMs also act as an important tool to study other cell behaviors, such as identifying specific cells,[103–106] improving enzyme probes,[107] revealing membrane dynamics,[108] enhancing molecular imaging,[109,110] and mimicking context-dependent signaling.[111]

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4.1 SASMs for identifying specific cells, improving enzyme probes, or revealing membrane dynamics

Cellular senescence is a natural process sustaining normal physiological functions of life.[112] However, excessive accumulation of senescent cells may result in some diseases, such as atherosclerosis and tissue dysfunction.[113,114] Thus, it is necessary to identify and remove senescent cells in a timely manner. Forming SASMs in senescent cells turns out to be a valid approach to identify and remove the cells. As shown in Fig. 10A and B, β-galactosidase, overexpressed in senescent cells, is able to catalyze the hydrolytic removal of β-galactose in **40**, a peptide containing a fluorophore, to generate **41**. Self-assembly of **41** results in nanofibers, which exhibit increased fluorescence intensity for identifying senescent HeLa cells (s-HeLa). When the concentration of **40** is at 400 μM, only approximately 30% of senescent endothelial cells (s-ECs) survive, as shown in Fig. 10C.[103]

Besides removing senescent normal cells, it is more urgent to eliminate senescent cancer cells. As shown in Fig. 10D and E, **43**, a peptide derivative similar to **40**, contains a fluorescent molecule that is able to generate reactive oxygen species (ROS). Upon β-galactosidase catalyzed deglycosylation, **43** turns into **45**, which self-assembles to enhance the fluorescence intensity for distinguishing s-HeLa from the wild type HeLa cells (n-HeLa). Moreover, upon light irradiation to generate ROS, the assemblies of **45** exhibit appreciable inhibitory activity against s-HeLa (see Fig. 10F).[104] In addition to identifying and inhibiting cancer cells, SASMs are also able to image[106] and inhibit tumors.[105]

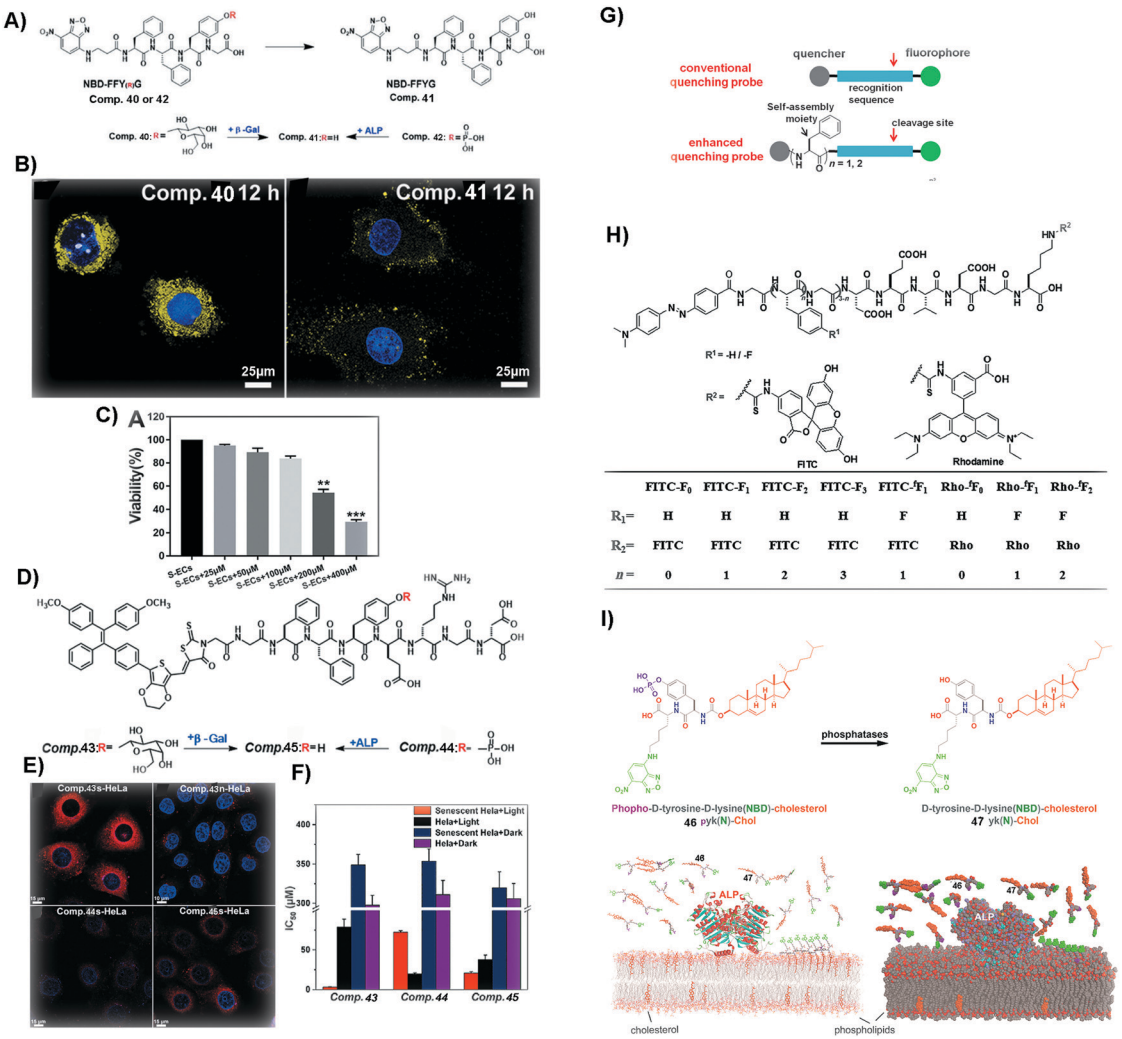

Fig. 10 (A) Molecular structures of **40–42**. (B) Images of senescent HeLa cells incubated with **40** and **41** (blue fluorescence from DAPI, representing the nucleus, and yellow from NBD, the N-terminal molecule). (C) Viability of s-ECs incubated with **40** at different concentrations. (A–C) Reproduced with permission from ref. 103. Copyright 2019, Royal Society of Chemistry. (D) Molecular structures of **43–45**. (E) Images of s-HeLa incubated with different compounds (**43–45**) and n-HeLa incubated with **43** (blue fluorescence from DAPI, representing the nucleus, and red from methoxy-substituted tetraphenylethene (TPE), the N-capping motif). (F) IC50 of HeLa cells incubated with different compounds (**43–45**). (G) Illustration of enhancing quenching of the self-assembled probe. (H) Molecular structures of different probes. (D–H) Reproduced with permission from ref. 104 and 107. (I) Molecular structures of **46** and **47** and the process of revealing the membrane dynamics by incubating **46** with cells. Adapted with permission from ref. 108. Copyright 2018, American Chemical Society.

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Although these methods are easy and effective, the background signal of some fluorescent probes could be too high. Thus, aggregation-causing SASMs are able to address this issue. As shown in Fig. 10H, the precursor, of which the N-terminal is dabcyl, a quencher, is a kind of traditional quenching probe. When caspases (apoptosis-associated enzymes) are present, the quencher is cleaved and the fluorescence intensity increases. To enhance the quenching effect of the precursors and lower the background signal, one approach is to incorporate different numbers of amino acids or amino acid derivatives, such as phenylalanine or 4-fluoro phenylalanine, which forms the compounds shown in Fig. 10H (n = 1/2/3). Assemblies of these compounds lower the background signals. Upon the addition of caspases, the fluorescence signal increases, which results in a high signal-to-noise ratio compared to that of traditional ones.[107]

Besides intracellular imaging, a method is still needed that is able to reveal the dynamics of the cell membrane. For example, as shown in Fig. 10I, compound **46**, containing 4-nitro-2,1,3-benzoxadiazole (NBD) and cholesterol, upon ALP catalysis forms molecule **47**. Assemblies of **47** are able to probe the nanoscale heterogeneity of membranes and reveal the membrane dynamics, thus providing a useful probe for understanding the dynamics of cell membranes.[108]

4.2 SASMs for imaging molecular self-assembly

SASMs are particularly useful to enhance molecular imaging for studying cell behavior. As shown in Fig. 11A and B, **48** conjugates with NBD to form compound **49**. After **49** enters into cells, under the catalysis of ALP, it loses the phosphate group to form **50**, which self-assembles to generate fluorescent nanofibers in nuclei, mitochondria and plasma membranes. This method, which is to image molecular self-assembly, is efficient to evaluate self-assembly and monitor the dynamics of these nanofibers.[109] In addition to imaging fluorescent molecule self-assembly, this method is also applicable for imaging non-fluorescent molecule self-assembly. For example, as shown in Fig. 11C, **51**, a non-fluorescent molecule, is able to co-assemble with a small amount of **53**, a compound that is similar to **49** but with the fluorescent molecule changed from NBD to dansyl (DNS). Adding 200 nM (lower than the critical micelle concentration) of **53** in cell culture results in fluorescence that is uniformly distributed. But on adding 200 nM of **53** and 500 μM (higher than the critical micelle concentration) of **51** in the cell culture, the fluorescence in the cells is localized (Fig. 11D). This observation proves that the assemblies are from **51**, not from **53**, confirming that this assay is able to image the self-assembly of non-fluorescent molecules. The result also suggests that the process of self-assembly may be through the protein secretion pathway, which is the endoplasmic reticulum to the Golgi to lysosomes/secretion.[110] These studies underscore SASMs as promising nanoprobes to enhance molecular imaging, which allows enzyme-triggered SASMs to be further developed as a unique approach for imaging cell behavior.[115,116]

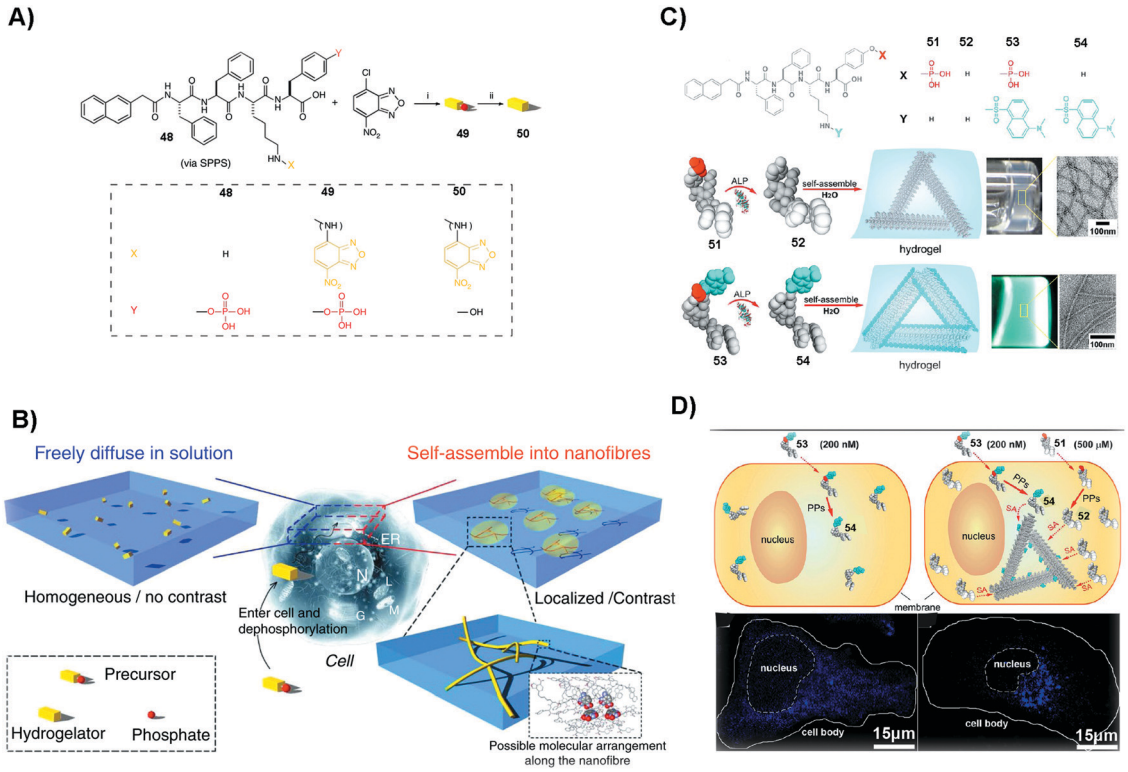

Fig. 11 (A) Molecular structures of **48–50**. (B) Illustration of molecular assembly. (A and B) Reproduced with permission from ref. 109. (C) Molecular structures of **51–54** and the corresponding hydrogels. (D) Images of HeLa cells incubated with different compounds (**53** or **51**/**53**). PP: protein phosphatase. (C and D) Adapted with permission from ref. 110. Copyright 2013, American Chemical Society.

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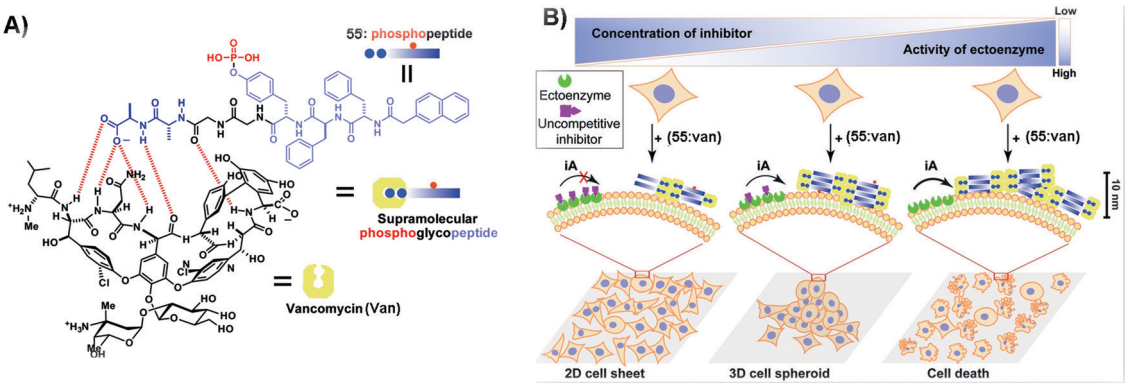

Fig. 12 (A) Illustration of **55** binding with Van. (B) SASMs mimicking context-dependent signaling. iA: instructed assembly. Reproduced with permission from ref. 111.

4.3 SASMs for mimicking context-dependent signaling

Context-dependent signaling plays an important role in many cellular functions. For example, in developing embryos, some WNTs (a protein family that secretes glycoproteins) can activate β-catenin to influence cell proliferation and survival, while other ones can modulate cellular movement by activating a β-catenin-independent signaling pathway.[117] Thus, using active matter such as SASMs to mimic context-dependent signaling is an effective method to understand the cell behavior. As shown in Fig. 12A, since vancomycin (Van) is an antibiotic that can specifically bind with D-Ala-D-Ala, the designed phosphopeptide **55** containing D-Ala-D-Ala is able to bind to Van, for inhibiting cancer cells. As shown in Fig. 12B, tissue-specific ALP (ALPL) on Saos-2 cells is able to catalyze the formation of the supramolecular complex (**55**:Van), generating nanofibers and resulting in cell death. An uncompetitive inhibitor can antagonize the activity of ectophosphatases (*e.g.*, ALPL). Thus, when adding this inhibitor to the cell culture, fewer nanofibers are generated in the cells and the cells survive and form 3D spheroids. More inhibitors are capable of resulting in the changes of the morphologies of the surviving cells, from 3D spheroids to 2D sheets.[111] The use of SASMs to mimic context-dependent signaling may help develop new approaches for studying cell behaviors.

5. Outlook

The past research on SASMs provides certain molecular and cellular understanding of the emergent properties of SASMs. Although SASMs show therapeutic effects on some diseases, many challenges still exist. For example, MDR in cancer and bacterial therapies still threatens human health. It is essential to increase the activities of SASMs to minimize the emergence of MDR. For instance, immunotherapy, a safe and efficient solution, is able to treat cancer with SASMs as the adjuvant.[118] The bacterial biofilm microenvironment might be a novel target to improve the efficacy of SASMs.[119] Applying SASMs in tissue engineering and regenerative medicine is at the beginning, but there is a concern of amyloidogenicity of aggregates. This issue requires careful kinetic control of the formation of SASMs to avoid inducing amyloid formation of endogenous biomolecules. Moreover, enhancing the activation of multipotent proteins is efficient to promote the use of SASMs in tissue engineering and regenerative medicine, such as in bone regeneration.[120] It is likely that the further understanding of enzymatic noncovalent synthesis in biology[24] will also help address current challenges of SASMs in disease treatment.

With the ability to design SASMs[121–123] and to determine the morphologies[124] and structures of SASMs,[125,126] it is time to explore the functions and applications of SASMs. While SASMs have exhibited a wide range of biological functions, the mechanistic study of SASMs remains insufficient. The elucidation of the molecular mechanisms of the biological functions of SASMs, undoubtedly, will contribute to the development of supramolecular assemblies for ultimately treating life-threatening diseases.

The small molecules used for forming SASMs have been largely centered on peptides, but the principles revealed by the study of SASMs should be applicable to other types of small molecules, such as nucleobases[127] or carbohydrates,[120] for discriminating the protein targets of supramolecular assemblies. Further research on SASMs will establish the knowledge foundation for engineering supramolecular assemblies to regulate protein–protein interaction networks[128] for controlling the fate of cells. In addition, the knowledge obtained in the research on SASMs will provide new insights for helping understand other challenging problems related to molecular self-assembly, such as the molecular mechanism of the aggregates of aberrant proteins that are considered as a plausible cause of neurodegenerative diseases (*e.g.*, Alzheimer's disease[129]). In addition, SASMs also represent a new direction of supramolecular chemical biology,[130] a promising field of chemistry that remains to be explored by molecular scientists.

Conflicts of interest

There are no conflicts of interest to declare.

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