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In Situ Construction of Functional Assemblies in Living Cells for Cancer Therapy

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Peptide-based materials hold great promise for various biomedical applications and have drawn increasing attention over the past five years. Despite the progress in fabrication and handling peptide materials in vitro, manipulating assemblies of peptides in living cells (or animals) is still in its infancy. In this contributing review, recent work is summarized using endogenous triggers to construct functional assemblies of peptides in vivo. After introducing the triggers for inducing peptide assemblies, the recent progress is highlighted of the in situ construction of assemblies for biomedical applications with emphasis on cancer therapy. Finally, a brief perspective is provided to discuss the future promises and challenges of this emerging area of supramolecular chemistry.

1. Introduction

Nature employs self-assembly to construct elaborate structures to fulfil various biofunctions with precise spatiotemporal control.[1,2] To give one striking example: as a major component of the cytoskeleton, higher-order structures of microtubules formed by polymerization of α- and β-tubulins, play crucial roles in many essential cellular processes, including cell movement, cell division, and cell proliferation.[3,4] In the last two decades, self-assembly served as a novel strategy for the synthesis of complex nanostructures for mimicking the properties of biological systems. One of the most attractive building blocks is the peptide, which has been widely explored in biomedical applications.[5–9]

As an essential biomolecule in nature, peptide holds several advantages, including excellent biocompatibility and biodegradability, ease of design and functionality, and low immunogenicity. Peptide-based biomaterials have already been widely used in biomedical applications and have shown great potential in tissue engineering, 2D and 3D cell culture, drug delivery, cancer therapy, and regenerative medicine. The related perspective in supramolecular biomaterials has already been covered by recent excellent reviews.[10–21] Despite these significant advances in biology and medicine, there is an increasing interest to construct peptide assemblies in situ of living cells and animals, for the applications of cancer therapy and imaging.[22–26] In the past decade, scientists have employed physiological stimuli to induce peptide self-assembly for constructing smart biomaterials in vitro. After the pioneering work that reported the concept of using endogenous alkali phosphatase (ALP)-instructed self-assembly of peptides for killing cancer cells,[27] considerable advances have been made along with the notion of in situ construction of peptide assemblies.

This review highlights the recent development of using cellular triggers to construct functional assemblies of the peptide in situ. This work mainly focuses on the recent work for cancer therapy, for peptide and polypeptide based biomaterials for biomedical imaging, the readers are encouraged to read the very recent excellent review.[28] We start with a less-discussed aspect of different triggers to induce peptide assemblies. Then we introduce the recent progresses of in situ construction of assemblies for biomedical applications with emphasis on cancer therapy. Finally, we provide a brief outlook to discuss future promises and challenges.

Various strategies have been reported in the literature to induce peptide self-assembly in vitro, including ionic modulation, heating-cooling, pH adjustment, metal ions induced coordination, light irradiation, redox reaction, and enzymatic reaction. Several compressive reviews have already discussed the strategies to induce self-assembly of peptidic molecules. The readers are recommended to consult those reviews.[6,7,16,23,29,30] Transferring the self-assembly of peptides in vitro into in vivo is a challenging task due to the complexity of living environment. The physiochemical difference between normal and cancer cells provides a hint for the rational design of in situ self-assembly of the peptides. After the first report of enzyme induced self-assembly (EISA) of peptide in HeLa cells,[27] the concept of "in vivo self-assembly" has been extended to other stimuli in the tumor microenvironment.[31]

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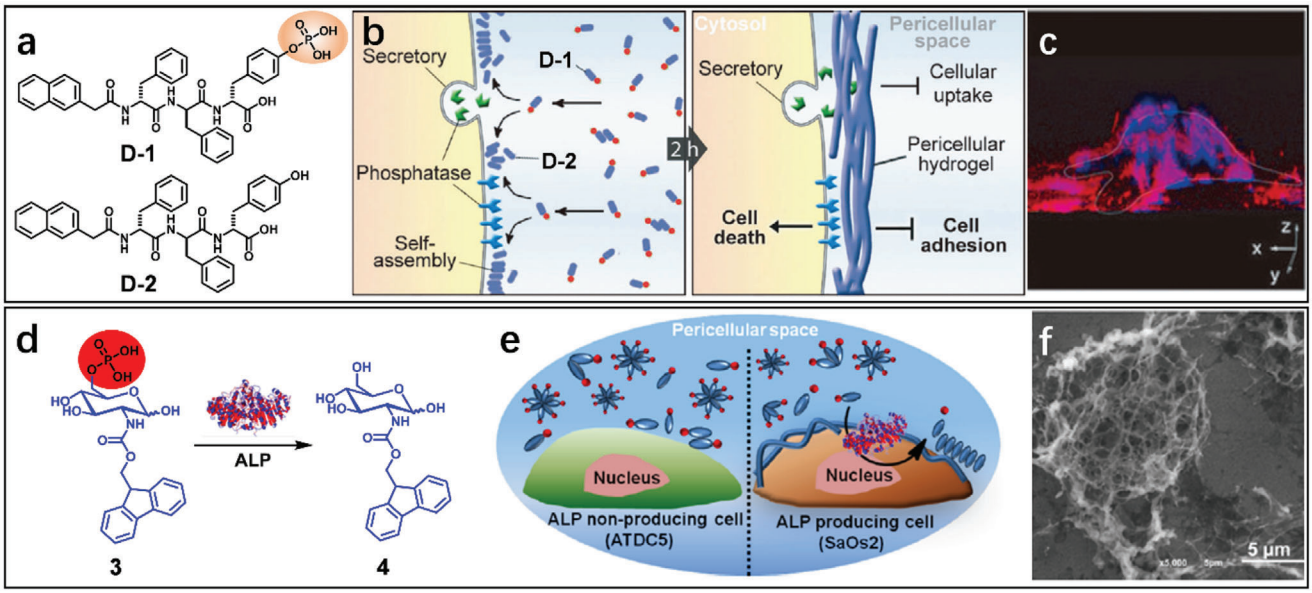

Figure 1. a) Molecular structure of D-1 and D-2; b) Enzyme-catalyzed formation of pericellular hydrogel/nanonets to induce cell death; c) CLSM image of HeLa cell treated by D-1. d) Enzymatic transformation from molecule 3 to 4 after addition of ALP; e) In situ biocatalytic self-assembly; f) SEM image of Saos-2 cells in the presence of 3. (a–c) Reproduced with permission.[32] Copyright 2014, Wiley-VCH GmbH. (d–f) Reproduced with permission.[34] Copyright 2015, American Chemical Society.

2. Enzymatic Pericellular Self-Assembly on Cell Surface

Cancer cells usually overexpress certain enzymes on the cell surface, the property of which has been utilized to construct peptide assemblies on cancer cell surface selectively for cancer therapy.

Xu and co-workers[32] reported the construction of pericellular hydrogel/nanonets to inhibit cancer cells. The small D-peptide **D-1** (Nap-$_D$-Phe-$_D$-Phe-$_D$-phospho-Tyr, **Figure 1**a) could transform into **D-2** after ALP catalyzed dephosphorylation, resulting in the formation of hydrogel through EISA in phosphate buffered saline (PBS). In pericellular space, the EISA could be triggered by surface and secretory phosphatases, leading to the formaton of pericellular hydrogel/nanonets selectively around the cancer cell that overexpressed phosphatases (e.g., HeLa, human uterine sarcoma cells (MES-SA/Dx5), Figure 1b,c). The cell-based experiment indicated the formed hydrogel/nanonets could interrupt the cellular mass change, leading to the apoptosis of cancer cells. This work offers a promising strategy for cell apoptosis in the cellular environment through in situ self-assembly of peptides.

Unlike the peptide-based precursors for self-assembly, carbohydrate derivatives have been emerging as an alternative build block.[33] Ulijn and co-workers[34] reported localized biocatalytic self-assembly and gelation on the membrane of osteoblasts to control the cell fate. The aromatic carbohydrate amphiphile (*N*-(fluorenylmethoxycarbonyl)-glucosamine-6-phosphate, **3**, Figure 1d) undergoes molecular structure change via enzymatic dephosphorylation to **4**, which then self-assemble into nanofibers in aqueous solution. Being incubated with ALP overexpressed osteosarcoma cell line (Saos-2), ALP on the cell surface initiated the self-assembly process of **4** (Figure 1e), resulted in the generation of hydrogels. The authors used confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) to examine the formation of hydrogel on cell surface (Figure 1f). Cell experiment indicated that the gelation process in the pericellular environment could induce cell death after 24 h. Moreover, the pericellular ALP induced gelation is a phosphatase concentration-dependent behavior, and the gelation was not observed in the lower ALP expressing cell line, such as prechondrocytes (ATDC5). This work extended the reservoir of build blocks for EISA to kill cancer cells selectively.

Besides using a single enzyme to induce in situ self-assembly, dual targeting provides more selectivity and specificity toward cancer cells. Zhao and Wang groups[35] reported a tumor-selective cascade activatable self-retained system (TCASS). The peptide **5** consists of four parts, a tumor-specific recognition motif (AVPIAQK), an enzyme cleavable linker (DEVD), a self-assembly motif (KLVFFAECG), and a functional molecule (cyanine dye, or doxorubicin) (**Figure 2**a). After incubating with cancer cells, the AVPIAQK sequence could specifically recognize the overexpressed X-linked inhibitor of apoptosis protein (XIAP), then activated the caspase-3/7 after the recognition process. The cleaved molecules rapidly self-assemble into nanofibers with β-sheets conformation through hydrogen bonding (Figure 2b). Compared with typical materials, the authors found that such a strategy exhibited the enhanced accumulation around tumor tissue, suggesting the TCASS effect. Moreover, the small peptide could be excreted from organs of the reticuloendothelial system, while remaining at tumor tissue. This work provides a novel strategy for tumor imaging and therapy by incorporating different functional groups.

Recently, Yang and co-workers[36] reported the EISA in the pericellular space of the cancer cells by dual targeting. The small peptide derivate **6** (NBD-GFFpYG-CCK6, **Figure 3**a) consists of two parts, a self-assembling sequence (NBD-GFFY) and a ligand

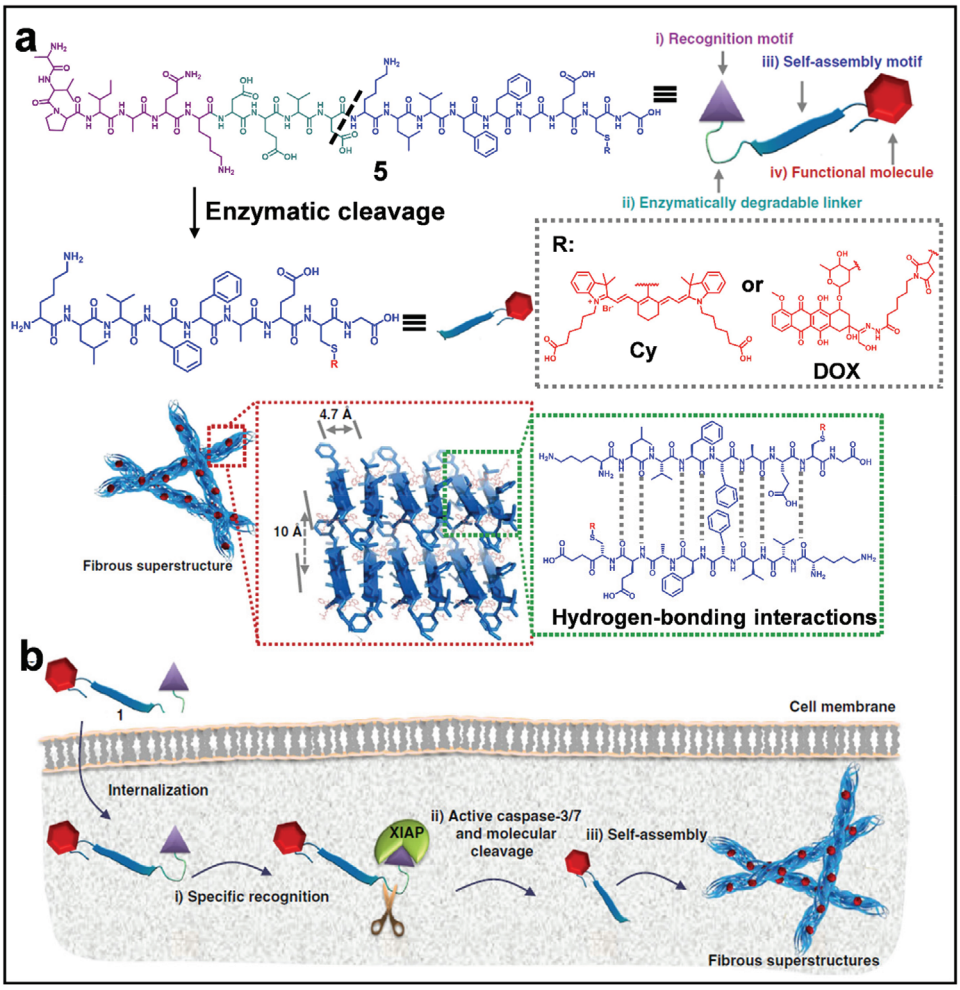

Figure 2. a) The molecular design and b) Scheme of tumor-selective cascade activatable self-detained system (TCASS). Reproduced with permission.[35] Copyright 2019, Nature Publishing Group.

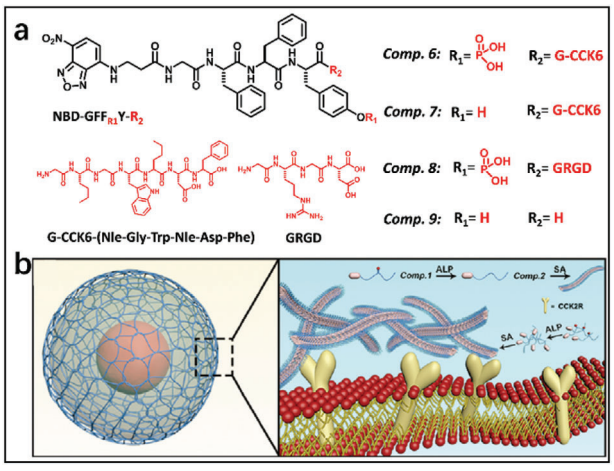

Figure 3. a) Molecular structures of precursor and the hydrogelator; b) Schematic illustration of **Comp. 6** self-assemble on the cell surface. Reproduced with permission.[36] Copyright 2019, The Royal Society of Chemistry.

of the human cholecystokinin-2 receptor (CCK2R) (Nle-Gly-Trp-Nle-Asp-Phe) overexpressing in the cancer cell membrane. Upon the enzymatic reaction induced by ALP, the **comp. 6** converts to **comp. 7**, then the receptor of CCK-6 interacts with CCK2R, resulting in the formation of hydrogels around the cancer cell (Figure 3b). The authors could only observe the formation of hydrogel in the cancer cells, which express a high level of extracellular ALP and membrane CCK2R (e.g., HeLa, human hepatocellular carcinomas cells (HepG2)). What is more, incubating **comp. 7**, **comp. 8**, and **comp. 9** with cancer cells hardly form the hydrogels, indicating the importance of CCK-6 in the hydrogel formation. The results highlight the importance of both EISA and overexpressed ligand of cancer cells in the formation of hydrogels, providing a strategy for in situ self-assembly around cancer cells precisely.

2.1. Intracellular Self Assembly

Compared with the formation of hydrogel on the cell surface, the intracellular formation of a hydrogel by small molecules

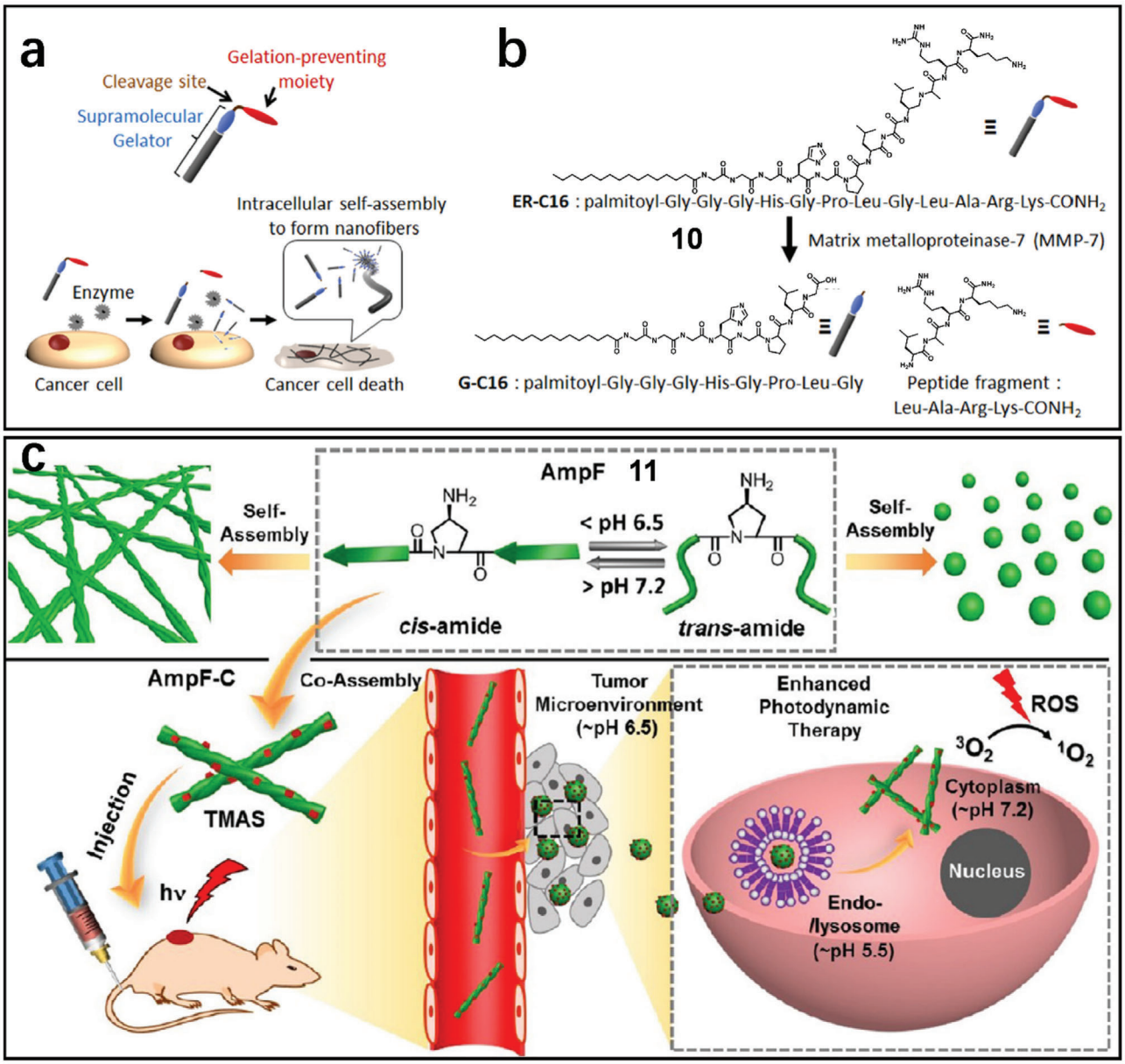

Figure 4. a) Cancer cell death induced by enzyme-responsive supramolecular self-assembly; b) Molecular structures of *N*-palmitoyl-Gly-Gly-Gly-His-Gly-Pro-Leu-Gly-Leu-Ala-Arg-Lys-$CONH_2$ (ER-C16, 10), *N*-palmitoyl-Gly-Gly-Gly-His-Gly-Pro-Leu-Gly (G-C16), and Leu-Ala-Arg-Lys-$CONH_2$. c) Schematic illustration of designed pH-responsive coassembly systems of peptides for nanomedicines. Reproduced with permission.[38,39] Copyright 2015 and 2019, American Chemical Society.

is more challenging. Since the most reported drug targets are inside cells, the intracellular formation of the hydrogel could minimize drug resistance and provide a strategy for increasing the activity of current chemotherapy.[37] Maruyama and co-workers[38] reported the cancer cell death induced by EISA of a supramolecular gelator (*N*-palmitoyl-Gly-Gly-Gly-His-Gly-Pro-Leu-Gly-Leu-Ala-Arg-Lys-$CONH_2$, **10**, **Figure 4**b). Peptide **10** contains four parts, the alkyl chain provides hydrophobic interaction in an aqueous solution, the sequence of Gly-Gly-Gly-His provides hydrogen bonds during the self-assembly process, and tetrapeptide (Pro-Leu-Gly-Leu) serves as the substrate of matrix metalloproteinase-7 (MMP-7). The cationic dipeptide of Arg-Lys could interrupt the formation of nanofibers before addition of enzyme. After incubating with cells, the ER-C16 could be hydrolyzed by MMP-7 to generate G-C16, which then self-assembles into the nanofibers in the intracellular space and influences the cellular function (Figure 4a). The author demonstrated that ER-C16 exhibited cytotoxicity against cancer cells while showed low toxicity toward normal cells. This study developed a new drug-like peptide assemblies based on supramolecular chemistry to realize cancer therapy.

Yu and co-workers[39] reported a novel kind of nanomedicine that responses to the tumor microenvironment through tumor microenvironment-adaptable self-assembly (TMAS) (Figure 4c).

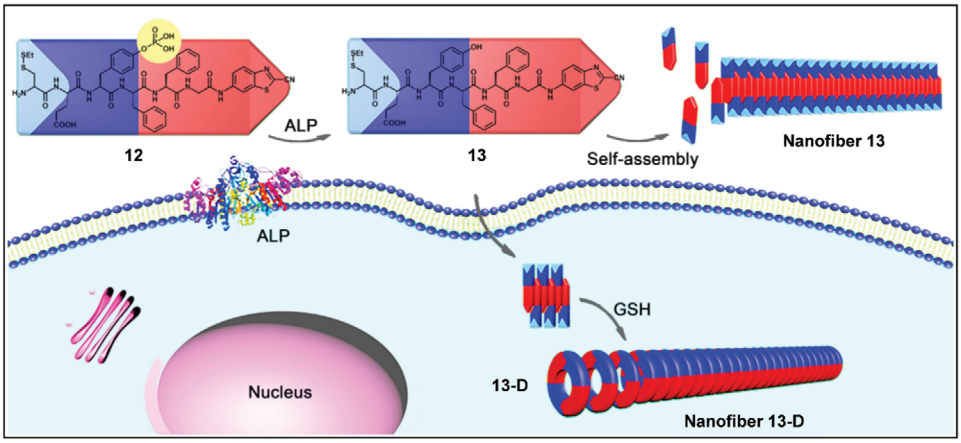

Figure 5. Molecular structures of compounds 12, 13, 13-D and the schematic illustration of hierarchical self-assembly in extra- and intracellular environment. Reproduced with permission.[40] Copyright 2016, American Chemical Society.

The pentapeptide (FF-Amp-FF, AmpF, **11**) consists of one Amp residue coupled with two diphenylalanine segment. The Amp motif of this peptide is a pH-sensitive group, which induces the cis/trans-amide isomerization under the neutral and acidic pH. In neutral or mild acidic condition, **11** forms superhelices or nanoparticles morphology, respectively. The authors constructed the TMAS system through co-assembly of **11** and photosensitizer chlorin e6 (Ce6) conjugated 11 (**11-C**). Being uptake by cells, the superhelices of TMAS first formed in blood vessels (pH 7.4) and then transform into nanoparticles through the endo/lysosome process, where the pH changes to about 6.5. Moreover, the nanofibers could be reformed within the cancer cells (pH 7.2–7.6), resulting in increased retention time and delivery efficiency. The TMAS system generates ROS more efficiently than a single Ce6. The cell experiment indicated that the TMAS showed the high biocompatibility before laser irradiation. After irradiation for 3 min, the cell viability of 4T1 breast cancer cells decreased rapidly due to the generation of ROS by TMAS. The TMAS system could offer a promising platform for combination therapy.

2.2. Dual Stimuli

Unlike the commonly used single trigger to induce self-assembly of small molecules in cells, employing two or more stimuli in spatiotemporal control is important and remains challenging. Liang and co-workers[40] used extracellular ALP and intracellular glutathione (GSH) to build different nanofibers in the cell environment. The precursor **12** (Cys(SEt)-Glu-Tyr-(H_2PO_3)-Phe-Phe-Gly-CBT, **Figure 5**) composes of an ALP-responsive peptide sequence (Tyr(H_2PO_3)-Phe-Phe), a disulfide cysteine motif, and a 2-cyano-6-aminobenzothiazole (CBT) group. Upon the dephosphorylation by extracellular ALP, **12** converts to **13** to form nanofiber in the pericellular space. When **13** was endocytosed into the intracellular environment, the GSH-initiated condensation through CBT motif could induce the transformation from **13** into cyclic dimer (**13**-D), leading to the generation of another kind of nanofiber. Compared with the hydrogel formed by **13**, the hydrogel formed by **13-D** exhibited an enhanced mechanical strength, indicating the stronger π–π interaction. The cell experiment also demonstrated that **12** could differentiate the cell environment and then self-assemble hierarchically into different kinds of nanofibers. This work provides a platform for regulating nanostructures by using a click condensation reaction.[41]

In another example, Yang and co-workers[42] used ALP and GSH as two tandem triggers to regulate the self-assembly of peptide in liver cancer cells. The peptide **14** (NBD-GFFpY-ss-ERGD, **Figure 6**a) is a dual stimuli-responsive precursor that could convert into **comp. 15** (NBD-GFFY-ss-ERGD) after the addition of ALP, resulting in the formation of uniform nanoparticles. **Comp. 15** could further transform into **comp. 16** (NBD-GFFY-thiol) in the presence of GSH, leading to the generation of hydrogels that consist of nanofibers (Figure 6b). Compared with normal cells, liver cancer cells express both a high level of extracellular ALP and intercellular GSH. Coincubating **comp. 14** with HepG2 cells for 0.5 h, the authors observed the formation of nanoparticles around cell surface. Notably, the nanoparticles transformed into nanofibers that located near the nuclear membranes after 4 h incubation. In another kind of liver cancer cell line QGY7703, the author also observed the dense nanofibers near the cell membrane. These results demonstrated that the tandem self-assembly of **comp. 14** occurs both in the extracellular and intracellular space of liver cancer cells. Liver cancer cells uptake the higher amount of **comp. 14** than other kinds of cancer cells and normal cells. The cell experiment demonstrated that the tandem self-assembly leads to the liver cancer cell death while innocuous to normal cells. This work provided a new concept of a tandem strategy for cancer therapy.

Based on the above study, Yang group[43] further used the tandem self-assembly to treat lung cancer cells by two enzymes. Lung cancer cells express high levels of extracellular ALP and intracellular reductase. The peptide **17** (Figure 6c) is a dual-trigger responsive precursor. The extracellular ALP could induce the molecular transformation from **comp. 17** to **comp. 18**, resulting in the formation of nanoparticles. Being uptake by cells, **comp. 18** could escape from the lysosome and accumulate in mitochondria because of the presence of the azo group. Furthermore, in the presence of reductase, **comp. 18** converts into **comp. 19** to generate nanofibers in mitochondria. The formation of nanofibers in mitochondria further disrupts the integrity of the mitochondria

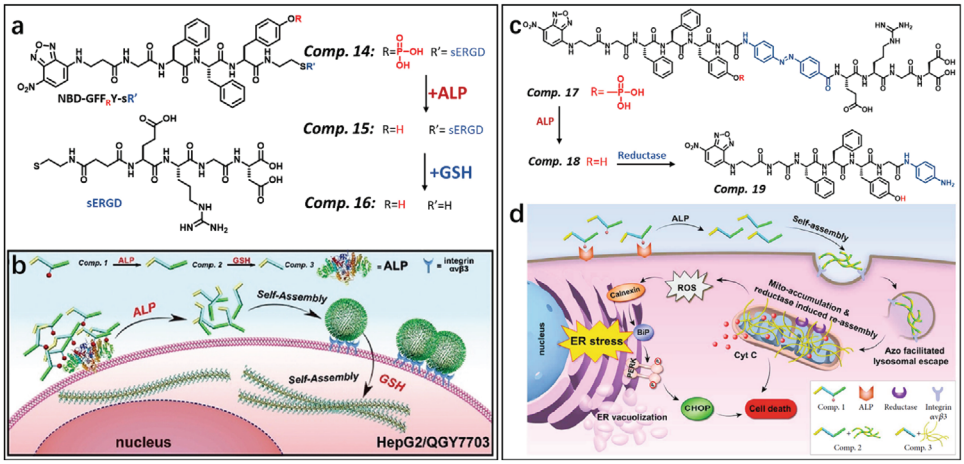

Figure 6. a) Molecular structures of the precursor, the ALP responsive and GSH responsive molecules; b) The tandem molecular self-assembly in the extra- and intra cellular environment of liver cancer cells. c) Molecular structures and enzymatic transformation of molecules; d) Schematic illustration of endoplasmic reticulum stress induced by the tandem molecular self-assembly. (a,b)Reproduced with permission.[42] Copyright 2018, American Association for the Advancement of Science (AAAS). (c,d) Reproduced with permission.[43] copyright 2019, AAAS.

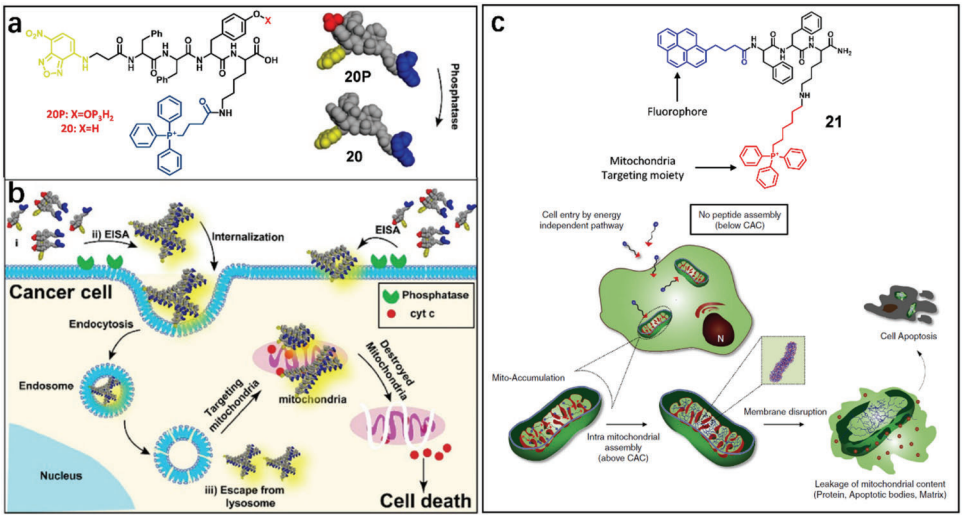

Figure 7. a) Molecular structure of the precursor and the hydrogelator; b) Schematic illustration of EISA for targeting mitochondria and inducing cancer cell death. Reproduced with permission.[44] Copyright 2016, American Chemical Society. c) Chemical structure of mitochondria-targeting peptide amphiphile and intramitochondrial assembly of Mito-FF. Reproduced with permission.[45] Copyright 2017, Nature Publishing Group.

membrane, inducing the release of cytochrome C (Cyt C) and the generation of reactive oxygen species (ROS). These changes together induce the endoplasmic reticulum (ER) stress of cancer cells, resulting in lung cancer cell death (Figure 6d). As a first example, this work employs two enzymes for tandem reaction of peptide assemblies, which provides a novel strategy for the selective recognition and treatment of lung cancer.

2.3. Subcellular Organelle Targeting

To improve the efficacy of the hydrogel against cancer cells, subcellular targeting is a promising strategy by accumulating peptide inside the confined space where the target is. Xu and co-workers[44] integrated enzymatic self-assembly of peptide and mitochondria targeting for selectively killing cancer cells. The designed peptide (NBD-FFpYK-TPP, **20P**) contains four parts, a self-assembling sequence (FFYK), an ALP responsive tyrosine phosphate (pY), an environment-sensitive fluorophore (NBD, 4-nitro-2, 1, 3-benzoxadiazole), and a mitochondria targeting motif (TPP) (**Figure 7**a). Upon addition of phosphate, **L-20P** (or **D-20P)** converts into **L-20** (**D-20)** to self-assemble into fibrous nanostructures, respectively. The cell cytotoxicity experiment indicated that **D-20P** exhibited lower IC_{50} than the corresponding L-enantiomer. CLSM images demonstrated that the self-assembly of both enantiomers could escape from the lysosome through caveolae/lipid raft-mediated endocytosis and then target mitochondria, resulting in cancer cell death (Figure 7b). The authors also found that the dysfunction of mitochondria could release Cyt C from mitochondria into cytoplasm, which further activated the

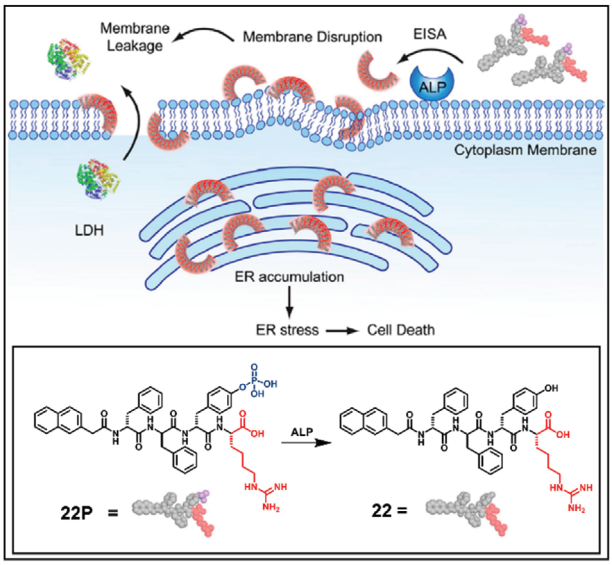

Figure 8. Schematic illustration of EISA to disrupt the cell membrane and target ER. Reproduced with permission.[46] Copyright 2018, American Chemical Society.

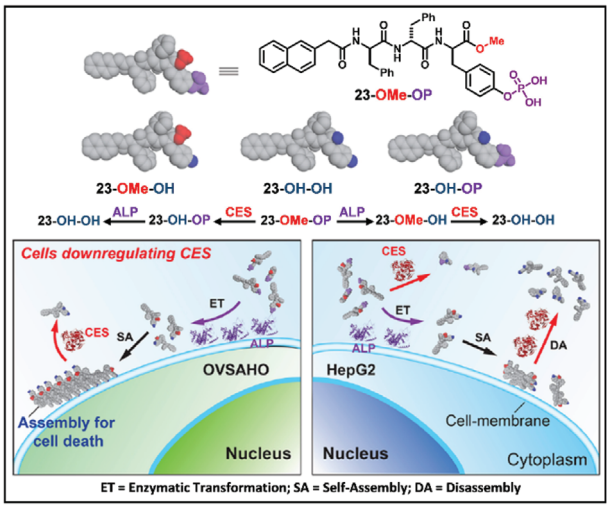

Figure 9. Molecular structure of precursor, the hydrolysis products, and the schematic illustration of targeting downregulation of cells for cancer therapy. Reproduced with permission.[47] Copyright 2017, American Chemical Society.

caspase cascade and induced cell death. More importantly, the authors demonstrated that the strategy could minimize the acquired resistance of cancer cells, one hallmark of cancer. Integration of the cell and subcellular targeting, this pioneering work offers a promising approach to minimize drug resistance of cancer.

In another contribution, Ryu and co-workers[45] reported the self-assembly of a peptide in mitochondria without assisting of enzymes to kill cancer cells. The molecule **21** (Figure 7c) could self-assemble into uniform nanofibers in PBS with a critical aggregation concentration (CAC) value of 60×10^{-6} M. Coincubating with HeLa cells, self-assembly of **21** could accumulate in mitochondria in a concentration-dependent manner. By modulating the initial concentration of peptide, the authors could control the amount of peptides inside mitochondria. To visualize the fibril formation in mitochondria, the authors synthesized **21-NBD**, an analogue of **21**. Coincubating **21** and **21-NBD** with HeLa cells, the authors observed the nanofibrils in mitochondria by CLSM and transmission electron microscopy (TEM). After incubating peptide with HeLa cells for 1 h, the authors observed severe damage of mitochondria that caused by the formation of nanofibers inside the mitochondria. The damage of the mitochondria membrane further induced the leakage of mitochondria contents to the cytosol, resulting in cell apoptosis (Figure 7d). This work illustrates the importance of CAC of peptide for subcellular targeting.

Endoplasmic reticulum (ER) is emerging as a novel target for cancer therapy, and selectively targeting the ER of cancer cells is limited. Very recently, Xu and co-workers[46] reported the selective cancer cell death that caused by enzymatic assembly of peptide for targeting ER. As shown in **Figure 8**, **22P** converts to **22** by ALP hydrolysis, leading to the formation of crescent-shaped aggregates on cell surface. Cytotoxicity experiments indicated that **22P** exhibit higher activity against cancer cells that overexpressing ALP. The Liposome cosedimentation assay and LDH releasing test showed that assemblies of **22** have an excellent membrane-binding affinity, which can break the cell membrane to release LDH into cell matrix. To visualize the distribution of assemblies, the author introduced a fluorescent molecule **F22P** by replacing the Nap group with the NBD group. CLSM images indicated that the **22P** and **F22P** peptides could accumulate on the ER to induce ER stress, resulting in cancer cell death. This study provides a promising approach to target ER for developing anticancer therapeutics.

2.4. Targeting Down-Regulation in Cancer Cells

The gain of functions (i.e., upregulation) and loss of functions (i.e., downregulation) are the two main aspects of cancer cells. The reported work mainly focused on the strategies for inhibiting upregulation. It is still a big challenge to target downregulation in cancer cells since it is independent from inhibition. The recent work by Feng et al.[47] reported a novel strategy for targeting downregulation in cancer cells. The EISA precursor **23-OMe-OP** contained both an ALP and carboxylesterase (CESs) responsive group. The ALP and CES can induce the molecular transformation from **23-OMe-OP** to **23-OMe-OH** and **23-OH-OP**, respectively (**Figure 9**). According to the measurement of critical micelle concentration (CMCs), the author obtained the self-assembly ability: **23-OMe-OH** > **23-OMe-OP** > **23-OH-OH** > **23-OH-OP**, which is corresponding to their cytotoxicity. Upon dephosphorylation of ALP, **23-OMe-OP** could transform into **23-OMe-OH** to self-assemble into nanofibrils. Meanwhile, the CES could cleave the ester bond and result in the disassembly of nanofibers. The author chose the cell lines that expressing ALP at a comparable level, while differed in CES expressing. For example, OVSAHO (an ovarian cancer cell line) downregulates the CES and HepG2 (hepatocyte cell) upregulates the CES. After incubating **23-OMe-OP** with two kinds of cells, the IC_{50} value of precursor toward HepG2 is 15 times higher than the OVSAHO cells, indicating **23-OMe-OP** could selectively target the

downregulation of CES in OVSAHO cells. This work offers a principle to target the downregulation of cancer cells for cancer therapy.

3. Perspective and Outlook

This review described a few promising examples of the rational design of in situ self-assembly for cancer therapy. The examples presented above underscore the opportunities and challenges that warrant further exploration of in situ self-assembly in vivo for biomedical applications. A deep understanding of the tumor microenvironment and intermolecular interactions of the hydrogelators (or small molecules) is crucial for achieving the designed functions. For example, utilizing the well-established difference between tumor and normal cells, such as pH gradient, ROS storage, overexpression of certain enzymes, and reducing environment in the cytoplasm, to design stimuli-responsive molecule is a direct strategy.[48–50] The reaction rate should be considered primarily before choosing one or more stimuli since the reaction-diffusion process plays a crucial role in determining the final functions (e.g., the final therapeutic efficiency). For example, the recent finding highlighted the importance of the enzymatic rate of small molecules for controlling cell fates (death or formation of 3D spheroids).[51,52] Besides, the reported assemblies' self-assembling motifs are almost discovered serendipitously, such as the well-developed dipeptide FF,[53] although it is originally from amyloid protein. Artificial intelligence-assisted self-assembly motif assessment and the corresponding bioactivity could be a new direction for high throughput screening to push this field much further.[54]

Despite the advances in this exciting field, there are still many challenges that have to be overcome, especially the following emerging issues. The most important one is to obtain direct evidence of in vivo self-assembly of small molecules. The current strategy employs bio-TEM of pretreated cells or tissues, which might be misled by intrinsic nanostructures of cells (or tissues). Rationally designing small molecules to form robust nanostructures in vivo is still challenging.[55] Using super-resolution fluorescence microscopy and nanocomputed tomography to study the nanostructures in vivo could be an ideal strategy.[56–59] The relationship between CMC and bioactivity of the supramolecular system is a crucial parameter for the efficiency of small molecules. The detailed analysis of the role of CMC (or self-assembly ability) and the corresponding bioactivity in vivo has shed light on the rational design of supramolecular therapies.[60,61] The lack of comprehensive quantitative analysis for cell death mechanisms induced by in vivo self-assembly is a gap for realizing such a strategy. Despite these challenges, we believe that the advances of knowledge from different disciplines will gradually solve these issues since their similarity to the challenges in living organisms.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

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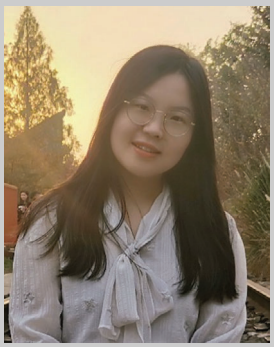
[1] G. M. Whitesides, B. Grzybowski, *Science* **2002**, *295*, 2418.
[2] B. A. Grzybowski, C. E. Wilmer, J. Kim, K. P. Browne, K. J. M. Bishop, *Soft Matter* **2009**, *5*, 1110.
[3] J. Howard, A. A. Hyman, *Nature* **2003**, *422*, 753.
[4] J. W. J. Kerssemakers, E. L. Munteanu, L. Laan, T. L. Noetzel, M. E. Janson, M. Dogterom, *Nature* **2006**, *442*, 709.
[5] J. D. Hartgerink, E. Beniash, S. I. Stupp, *Science* **2001**, *294*, 1684.
[6] H. G. Cui, M. J. Webber, S. I. Stupp, *Biopolymers* **2010**, *94*, 1.
[7] J. Shi, X. Du, D. Yuan, R. Haburcak, D. Wu, N. Zhou, B. Xu, *Chem. Commun.* **2015**, *51*, 4899.
[8] M. Sarikaya, C. Tamerler, A. K. Y. Jen, K. Schulten, F. Baneyx, *Nat. Mater.* **2003**, *2*, 577.
[9] S. G. Zhang, *Nat. Biotechnol.* **2003**, *21*, 1171.
[10] M. C. Branco, J. P. Schneider, *Acta Biomater.* **2009**, *5*, 817.
[11] M. P. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **2005**, *23*, 47.
[12] G. Fichman, E. Gazit, *Acta Biomater.* **2014**, *10*, 1671.
[13] J. B. Matson, R. H. Zha, S. I. Stupp, *Curr. Opin. Solid State Mater. Sci.* **2011**, *15*, 225.
[14] D. M. Ryan, B. L. Nilsson, *Polym. Chem.* **2012**, *3*, 18.
[15] J. P. Jung, J. Z. Gasiorowski, J. H. Collier, *Biopolymers* **2010**, *94*, 49.
[16] A. Altunbas, D. J. Pochan, in *Peptide-Based Materials*, Vol. *310* (Ed: T. Deming), **2012**, p. 135.
[17] X.-Q. Dou, C.-L. Feng, *Adv. Mater.* **2017**, *29*, 1604062.
[18] H. Shigemitsu, I. Hamachi, *Acc. Chem. Res.* **2017**, *50*, 740.
[19] S. Toksoz, M. O. Guler, *Nano Today* **2009**, *4*, 458.
[20] A. N. Moore, J. D. Hartgerink, *Acc. Chem. Res.* **2017**, *50*, 714.
[21] H. Wang, Z. Yang, *Nanoscale* **2012**, *4*, 5259.
[22] H. Wang, Z. Feng, B. Xu, *Angew. Chem., Int. Ed. Engl.* **2019**, *58*, 10423.
[23] G.-B. Qi, Y.-J. Gao, L. Wang, H. Wang, *Adv. Mater.* **2018**, *30*, 1703444.
[24] T. J. Ji, Y. P. Ding, Y. Zhao, J. Wang, H. Qin, X. M. Liu, J. Y. Lang, R. F. Zhao, Y. L. Zhang, J. Shi, N. Tao, Z. H. Qin, G. J. Nie, *Adv. Mater.* **2015**, *27*, 1865.
[25] K. M. Cheng, Y. P. Ding, Y. Zhao, S. F. Ye, X. Zhao, Y. L. Zhang, T. J. Ji, H. H. Wu, B. Wang, G. J. Anderson, L. Ren, G. J. Nie, *Nano Lett.* **2018**, *18*, 3250.
[26] Z. Feng, X. Han, H. Wang, T. Tang, B. Xu, *Chem* **2019**, *5*, 2442.
[27] Z. Yang, K. Xu, Z. Guo, Z. Guo, B. Xu, *Adv. Mater.* **2007**, *19*, 3152.
[28] M. Lv, E. Jan Cornel, Z. Fan, J. Du, *Adv. Biomed. Res.* **2021**, *1*, 2000109.
[29] R. V. Ulijn, A. M. Smith, *Chem. Soc. Rev.* **2008**, *37*, 664.
[30] M. de Loos, B. L. Feringa, J. H. van Esch, *Eur. J. Org. Chem.* **2005**, *2005*, 3615.
[31] Z. Feng, T. Zhang, H. Wang, B. Xu, *Chem. Soc. Rev.* **2017**, *46*, 6470.
[32] Y. Kuang, J. Shi, J. Li, D. Yuan, K. A. Alberti, Q. Xu, B. Xu, *Angew. Chem., Int. Ed. Engl.* **2014**, *53*, 8104.
[33] L. S. Birchall, S. Roy, V. Jayawarna, M. Hughes, E. Irvine, G. T. Okorogheye, N. Saudi, E. De Santis, T. Tuttle, A. A. Edwards, R. V. Ulijn, *Chem. Sci.* **2011**, *2*, 1349.
[34] R. A. Pires, Y. M. Abul-Haija, D. S. Costa, R. Novoa-Carballal, R. L. Reis, R. V. Ulijn, I. Pashkuleva, *J. Am. Chem. Soc.* **2015**, *137*, 576.
[35] H. W. An, L. L. Li, Y. Wang, Z. Wang, D. Hou, Y. X. Lin, S. L. Qiao, M. D. Wang, C. Yang, Y. Cong, Y. Ma, X. X. Zhao, Q. Cai, W. T. Chen, C. Q. Lu, W. Xu, H. Wang, Y. Zhao, *Nat. Commun.* **2019**, *10*, 4861.
[36] Y. Wang, J. Zhan, Y. Chen, S. Ai, L. Li, L. Wang, Y. Shi, J. Zheng, Z. Yang, *Nanoscale* **2019**, *11*, 13714.

[37] L. Rajendran, H.-J. Knoelker, K. Simons, *Nat. Rev. Drug Discovery* **2010**, *9*, 29.
[38] A. Tanaka, Y. Fukuoka, Y. Morimoto, T. Honjo, D. Koda, M. Goto, T. Maruyama, *J. Am. Chem. Soc.* **2015**, *137*, 770.
[39] M. Li, Y. Ning, J. Chen, X. Duan, N. Song, D. Ding, X. Su, Z. Yu, *Nano Lett.* **2019**, *19*, 7965.
[40] Z. Zheng, P. Chen, M. Xie, C. Wu, Y. Luo, W. Wang, J. Jiang, G. Liang, *J. Am. Chem. Soc.* **2016**, *138*, 11128.
[41] G. Liang, H. Ren, J. Rao, *Nat. Chem.* **2010**, *2*, 54.
[42] J. Zhan, Y. Cai, S. He, L. Wang, Z. Yang, *Angew. Chem., Int. Ed. Engl.* **2018**, *57*, 1813.
[43] D. Zheng, Y. Chen, S. Ai, R. Zhang, Z. Gao, C. Liang, L. Cao, Y. Chen, Z. Hong, Y. Shi, L. Wang, X. Li, Z. Yang, *Research (Washington, DC, USA)* **2019**, *2019*, 4803624.
[44] H. Wang, Z. Feng, Y. Wang, R. Zhou, Z. Yang, B. Xu, *J. Am. Chem. Soc.* **2016**, *138*, 16046.
[45] M. T. Jeena, L. Palanikumar, E. M. Go, I. Kim, M. G. Kang, S. Lee, S. Park, H. Choi, C. Kim, S. M. Jin, S. C. Bae, H. W. Rhee, E. Lee, S. K. Kwak, J. H. Ryu, *Nat. Commun.* **2017**, *8*, 26.
[46] Z. Feng, H. Wang, S. Wang, Q. Zhang, X. Zhang, A. A. Rodal, B. Xu, *J. Am. Chem. Soc.* **2018**, *140*, 9566.
[47] Z. Feng, H. Wang, R. Zhou, J. Li, B. Xu, *J. Am. Chem. Soc.* **2017**, *139*, 3950.
[48] P. P. He, X. D. Li, L. Wang, H. Wang, *Acc. Chem. Res.* **2019**, *52*, 367.
[49] R. Mo, Z. Gu, *Mater. Today* **2016**, *19*, 274.
[50] N. Q. Gong, Y. X. Zhang, X. C. Teng, Y. C. Wang, S. D. Huo, G. C. Qing, Q. K. Ni, X. L. Li, J. J. Wang, X. X. Ye, T. B. Zhang, S. Z. Chen, Y. J. Wang, J. Yu, P. C. Wang, Y. L. Gan, J. C. Zhang, M. J. Mitchell, J. H. Li, X. J. Liang, *Nat. Nanotechnol.* **2020**, *15*, 1053.
[51] H. M. Wang, J. F. Shi, Z. Q. Q. Feng, R. Zhou, S. Y. Wang, A. A. Rodal, B. Xu, *Angew. Chem., Int. Ed. Engl.* **2017**, *56*, 16297.
[52] H. M. Wang, Z. Q. Q. Feng, B. Xu, *J. Am. Chem. Soc.* **2019**, *141*, 7271.
[53] M. Reches, E. Gazit, *Science* **2003**, *300*, 625.
[54] P. W. Frederix, G. G. Scott, Y. M. Abul-Haija, D. Kalafatovic, C. G. Pappas, N. Javid, N. T. Hunt, R. V. Ulijn, T. Tuttle, *Nat. Chem.* **2015**, *7*, 30.
[55] Z. Feng, H. Wang, F. Wang, Y. Oh, C. Berciu, Q. Cui, E. H. Egelman, B. Xu, *Cell Rep. Phys. Sci.* **2020**, *1*, 100085.
[56] L. Albertazzi, D. van der Zwaag, C. M. A. Leenders, R. Fitzner, R. W. van der Hofstad, E. W. Meijer, *Science* **2014**, *344*, 491.
[57] Q. X. Yao, C. L. Wang, M. F. Fu, L. R. Dai, J. B. Li, Y. Gao, *ACS Nano* **2020**, *14*, 4882.
[58] M. M. Zhang, Y. Guan, Z. Dang, P. G. Zhang, Z. Zheng, L. Chen, W. Kuang, C. C. Wang, G. L. Liang, *Sci. Adv.* **2020**, *6*, eaba3190.
[59] M. Kumar, J. Y. Son, R. H. Huang, D. Sementa, M. Lee, S. O'Brien, R. V. Ulijn, *ACS Nano* **2020**, *14*, 15056.
[60] Z. Q. Q. Feng, H. M. Wang, X. Y. Chen, B. Xu, *J. Am. Chem. Soc.* **2017**, *139*, 15377.
[61] H. Su, F. H. Wang, W. Ran, W. J. Zhang, W. B. Dai, H. Wang, C. F. Anderson, Z. Y. Wang, C. Zheng, P. C. Zhang, Y. P. Li, H. G. Cui, *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 4518.



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