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Lysosomal Peptide Self-Assembly to Control Cell Behavior

Sangshuang Li[a] and Huaimin Wang*[a]

Lysosomes are membrane-enclosed organelles that play key roles in degrading and recycling cellular debris, cellular signaling, and energy metabolism processes. Confinement of amphiphilic peptides in the lysosome to construct functional nanostructures through noncovalent interactions is an emerging approach to tune the homeostasis of lysosome. After briefly introducing the importance of lysosome and its functions, we discuss the advantages of lysosomal nanostructure formation for disease therapy. We next discuss the strategy for triggering the self-assembly of peptides in the lysosome, followed by a concise outlook of the future perspective about this emerging research direction.

Introduction

Lysosomes are essential membrane-enclosed organelles in eukaryotic cells, often referred to as the cell's "waste disposal system" because of their pivotal role in degrading and recycling cellular waste.[1] Packed with over 60 diverse hydrolytic enzymes, lysosomes can break down macromolecules, damage cell components, and engulf particles by the hydrolytic enzymes in their acidic lumen.[2] Lysosomes can digest proteins, lipids, carbohydrates, and nucleic acids, liberating their constituent monomers such as amino acids, fatty acids, sugars, and nucleotides for reuse in cellular metabolism, thus ensuring the efficient turnover of cellular materials[3] and preventing potentially harmful buildup within cells.[1c] Besides, they can control cellular components through the selective degradation of aberrant or misfolded proteins to decrease the risk of proteostatic imbalances.[4] Beyond intracellular regulation, lysosomes are instrumental in the degradation of internalized pathogens and cellular signaling. Dysfunction of lysosomes can lead to the accumulation of waste, contributing to various diseases such as lysosomal storage disorders.[1a] The multifaceted functions of lysosomes underscore their indispensability to cellular physiology, which has been implicated in many diseases, including cancer progression. With a better understanding of the function and dysfunction of the lysosome, selective targeting of the lysosome has been suggested as a novel opportunity for treating diseases.[5]

Self-assembly is an ingenious strategy to make functional structures in nature, which refers to the process by which molecules spontaneously organize into structured aggregates or ordered conformations. This process relies on noncovalent interactions (e.g., hydrogen bonding, hydrophobic interactions, and electrostatic forces) between building blocks including peptides, amino acid derivatives, and sugars.[6] The acidic environment in lysosomes and some of the highly expressed proteases can serve as a trigger to initiate self-assembly. Recent developments in peptide self-assemblies have revealed that controlling the self-assembly of peptides in lysosomes can manipulate cellular behavior and be applied to treating diseases, including cancer and aging. For example, lysosomal self-assembly of peptides can tune the membrane permeability of the lysosome, resulting in redirecting functional molecules from the lysosome and fulfilling its function in the place of the target. In addition, lysosomal membrane permeabilization (LMP) can induce a type of apoptosis pathway that can bypass the classical caspase-dependent cell death, which could be used to kill cancer cells selectively.

This concept will mainly focus on the recent development in the lysosomal nanostructure formation of self-assembled peptides for controlling cell behaviors. We arrange this concept in the following manner: Firstly, we introduce the strategy of using the acidic condition of lysosomes to trigger self-assembly. Then, we describe methods to trigger the self-assembly of peptides directly and indirectly by enzymes in the lysosome. Finally, we give a concise outlook of its future perspective on this emerging research direction.

Using Acidic pH of Lysosome to Trigger Self-Assembly

Using the acidic environment of lysosomes to trigger the self-assembly of peptides is a promising approach in nanomedicine. Lysosomes have a pH ranging from 4.5 to 5.0, which is lower than the pH of the cytosol and other cellular organelles.[7] By rationally designing pH-responsive peptides, the proton in the lysosomal lumen can be utilized to achieve the targeting of the self-assembly of peptides in lysosomes to result in higher-order assemblies. Specifically, pH-sensitive groups undergo cleavage under acidic conditions to change the side chain's charge status by protonation, which significantly increases the intermolecular interaction among peptides through non-covalent interactions. Meanwhile, these changes could also result in a conformational change to facilitate the formation of ordered aggregation of peptides.[8]

Inspired by the pH-responsive natural protein, Wang and co-workers chose Val-Glu-Ala-Leu-Tyr-Leu (VEALYL), a segment

[a] S. Li, Dr. H. Wang
Department of Chemistry
School of Science, Westlake University
No. 600 Yungu Road, 310030 Hangzhou, Zhejiang Province, China
E-mail: wanghuaimin@westlake.edu.cn

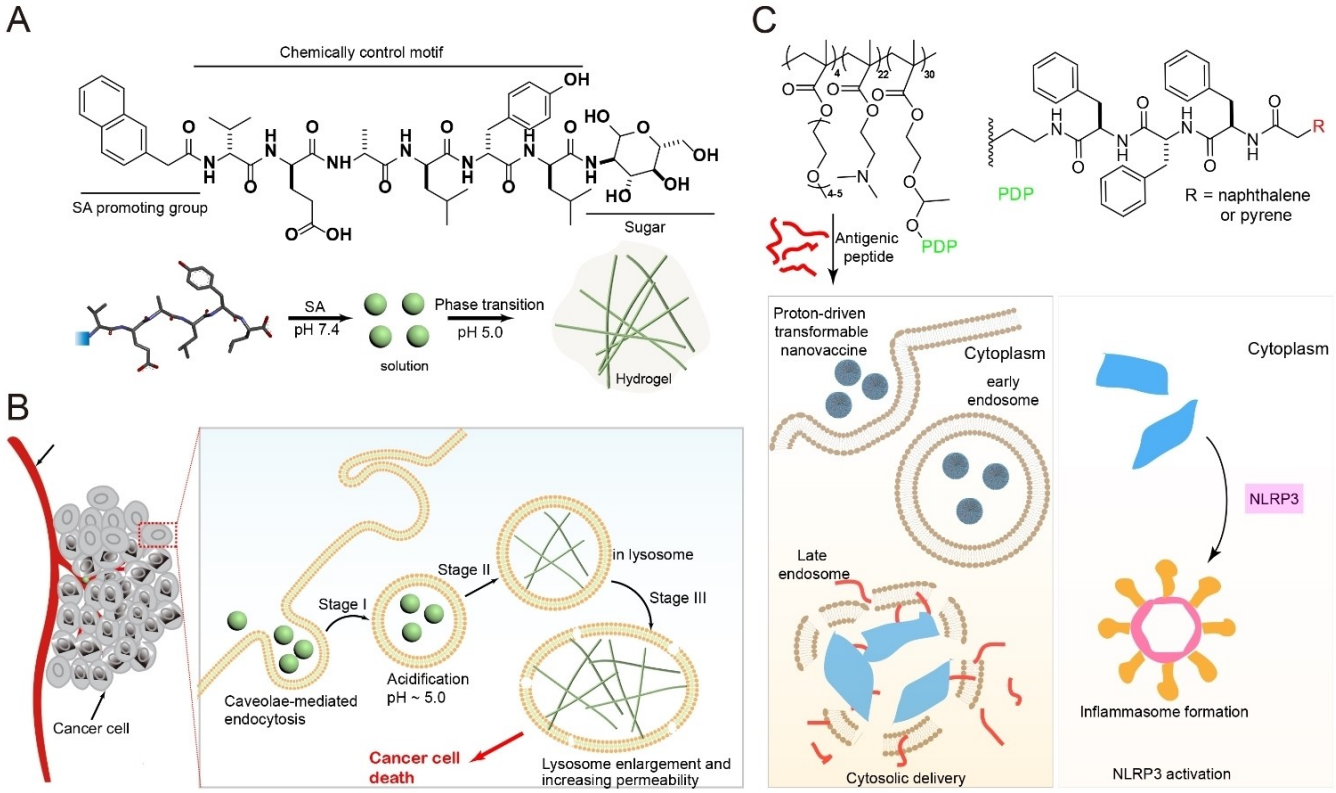

Figure 1. Strategies of using acidic pH of lysosome to trigger self-assembly. A) Molecular structure of LTP composed of a self-assembly (SA) promoting group, a pH transformable motif and C-terminal modification with a sugar. B) Phase-transformation process of LTP in living cells. After being uptake by cancer cells through endocytosis (stage I), oligomers of LTP accumulate in the lysosomes and transform into nanofibrous hydrogel through proton-induced phase transformation (stage II). The nanofibrous hydrogel further induces the enlargement of the lysosome (stage III) and causes LMP to result in cancer cell death. Reproduced with permission.[9] Copyright 2021, Wiley. C) Schematic illustration of a proton-driven NTV for cancer immunotherapy. The NTV is composed of a polymer–peptide conjugate-based NT loaded with AP. The NTV has a spherical morphology with a diameter of ~100 nm at pH 7.4. After the NTV is internalized by DCs, the acidic endosomal environment (pH 5.6) will trigger fast cleavage of the PDP peptide, which will then re-assemble into nanosheets with sizes in the range of 5–8 μm. The morphological change leads to disruption of the endosomal membrane and delivery of AP into the cytosol. Moreover, the cytoplasmic nanosheets activate the NLRP3 inflammasome pathway, promoting DC maturation and antigen processing. These two features contribute to the enhanced cross-presentation of AP to $CD8^+$ T-cells and efficient antitumor immunity. PDP, pyrene-conjugated D-peptide; NLRP3, NOD-like receptor, pyrin-domain-containing.

of human insulin protein with pH-responsive property, to conjugate aromatic group at its N-terminal and sugar group at its C-terminal to form aromatic-motif-appended pH-responsive hexapeptide (LTP) (Figure 1A). They found that LTP forms amyloid-like fibrils with β-sheet structures under the acidic environment in the lysosome.[9] After being uptake by cancer cells, LTP can accumulate in lysosomes and be transformed into nanofibrous biomolecular condensates expanded in lysosomes through a proton-induced phase transition. The formed condensates could increase the volume of the lysosome, resulting in LMP (Figure 1B). The authors also demonstrated that the lysosome expanding strategy in this work could re-direct the weakly hydrophobic drugs escaping from the lysosome to reach the target for addressing multi-drug resistance.

Sangshuang Li received a B.S. in Engineering from the College of Nano Science & Technology, Soochow University, China. She is currently a Ph.D. student in the group of Professor Huaimin Wang at Westlake University. Her research interests include the design of functional peptides for tumor immunotherapy.

Huaimin Wang, after receiving his B.S. from Tianjin University, Huaimin obtained his Ph.D. from Nankai University under the supervision of Professor Zhimou Yang. Before starting his independent research at Westlake University, he was a postdoctoral fellow with Prof. Bing Xu at Brandeis University. His research interests mainly focus on applying supramolecular assemblies of small molecules to explore and engineer living organisms; designing functional supramolecular nanomaterial for applications in materials chemistry and biomedicine.

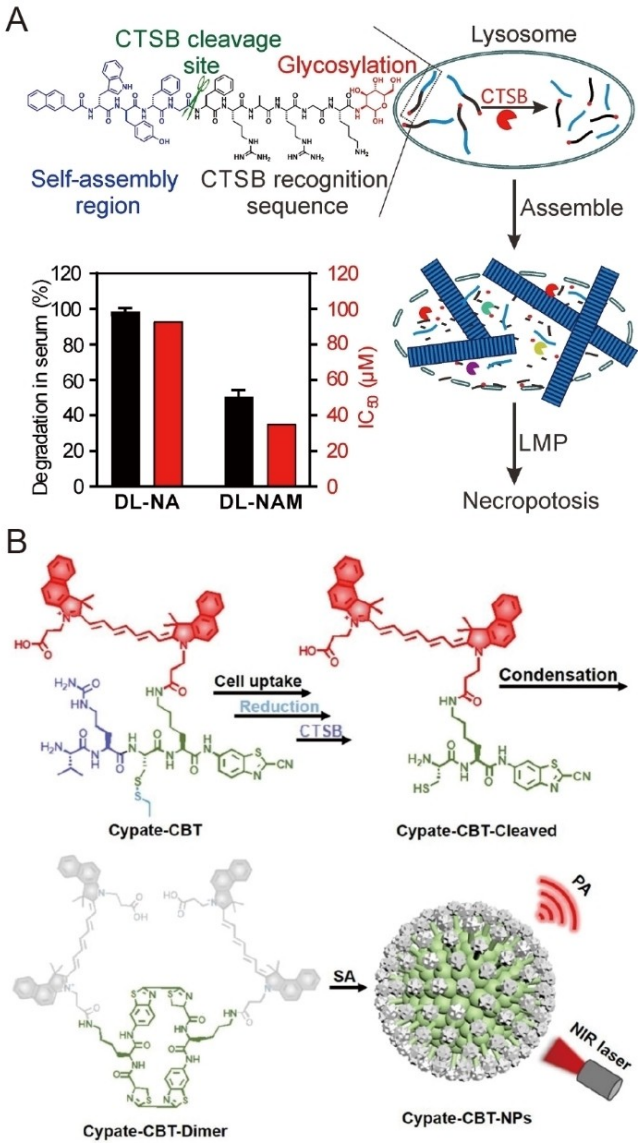

Figure 2. Strategies of using enzyme in lysosome to trigger self-assembly in a direct way. A) Illustration of CTSB-responsible self-assembly peptides and representation of the formation of functional higher-order assemblies in living cells for selective cancer cell inhibition. Reproduced with permission.[13] Copyright 2021, Wiley. B) CTSB-triggered self-assembly of Cypate-CBT-NPs for photoacoustic imaging of CTSB activity in vitro and in vivo. Reproduced with permission.[15] Copyright 2022, Wiley.

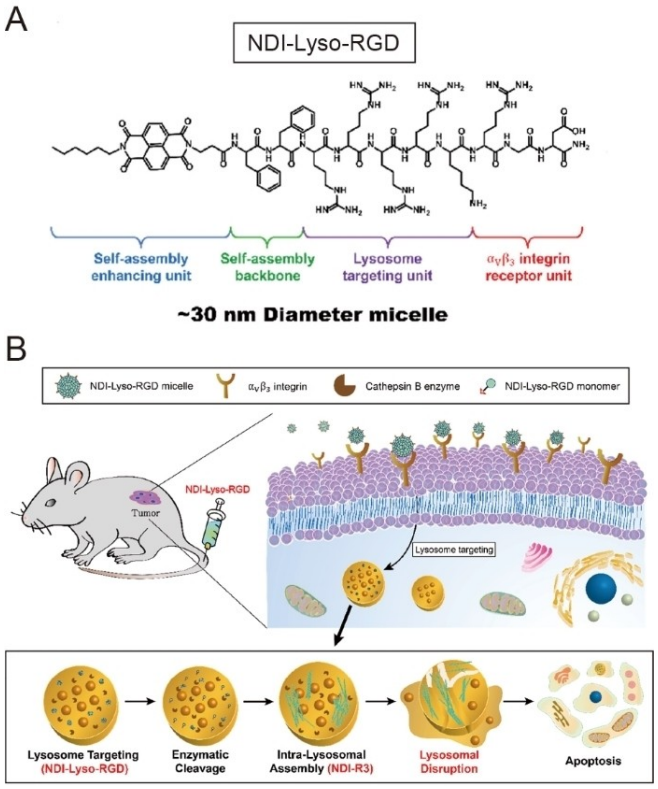

Figure 3. A strategy of using enzyme in lysosome to trigger self-assembly in a direct way. A) Molecular Structure of the Peptide Amphiphile (NDI-Lyso-RGD). B) Schematic illustration of lysosome-targeted self-assembly for cancer therapy. Reproduced with permission.[16] Copyright 2023, American Chemical Society.

Lysosomal trapping of tumor vaccines limits the efficiency of immunotherapy. To overcome this challenge, Liang's group reported a proton-driven nanotransformer-based vaccine (NTV) by designing a polymer–peptide conjugate that could undergo morphology transformation through the change of pH.[10] The amphiphilic polymer–peptide conjugate forms spherical nanostructures at pH 7.4, which could re-assemble into nanofibers or nanosheets at pH 5.6. The authors conjugated naphthalene-conjugated D-peptide (NDP) or pyrene-conjugated D-peptide (PDP) to p(DMAEMA$_{22}$-OGEMA$_4$)-b-p(MAVE)$_{30}$ via an acetal bond. The acetal bond could be rapid cleavage and release of the peptides under an acidic environment (pH 5.6). Interestingly, PDP forms nanosheets rather than nanofibers that are usually observed in NDP system. The nanosheets formation could boost tumour immunity via activation of specific inflammation pathways. This transformation changes the lysosomal membrane permeability, allowing antigen peptides to escape from lysosomes to achieve cytoplasmic delivery via acidic environment-mediated peptide self-assembly, thereby promoting the maturation of dendritic cells and facilitating later activation of CD 8+ T cells. This proton-driven self-assembly delivery platform offers an efficient strategy for cancer immunotherapy, thus providing new insights into the strategy of using the acidic pH of lysosome to trigger self-assembly.

Using Lysosomal Enzyme to Trigger Self-Assembly

In biological systems, biocatalytic reactions carried out by enzymes enable the processing of molecules to transform into natural substrates. Therefore, enzyme-specific recognition motifs could be incorporated into the molecular scaffold of precursors. Proteases catalyze the hydrolysis of peptide bonds, and thus, they are expected to be effective tools for biologically responsive activation of peptide self-assembly.[11] The diversity and specificity of proteases in terms of expression levels in

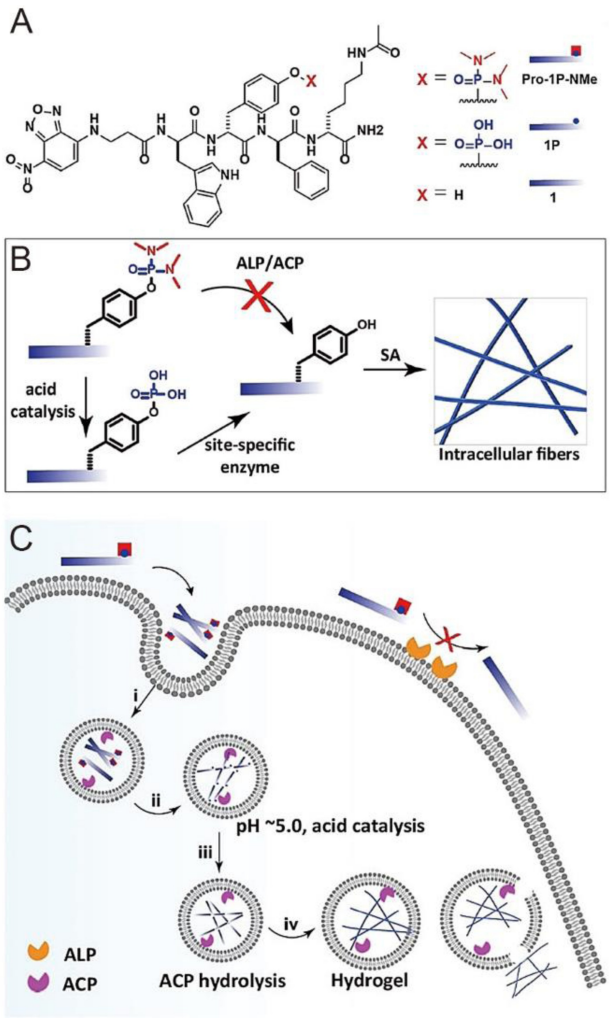

Figure 4. A strategy of using enzyme in lysosome to trigger self-assembly indirectly. A) Molecular structures and B) illustration of multistage chemical reactions in vitro. C) Illustration of the site-specific construction of nanofibers in living cells through multistage processes: i) cellular uptake of molecules; ii) acid-catalyzed hydrolysis of the P–N bond of Pro-1PNMe results in 1P which forms oligomers; iii) acid phosphatase in the lysosome further induced hydrolysis of 1P to 1; and iv) self-assembly of 1 into an entangled nanofibrous network. Reproduced with permission.[20] Copyright 2021, Wiley.

different cell types and organelles, as well as in terms of reaction substrates and cleavage sites, provide the basis for enzyme-directed spatiotemporal programming of peptide self-assembly.[12] Among the numerous enzymes in lysosomes, cathepsin B (CTSB) has attracted increased attention because of its overexpression in solid tumors.

Drawing inspiration from enzyme-instructed self-assembly (EISA) and CTSB, the Wang group rationally designed a series of structural analogs of peptide precursors containing the enzymatic cleavage site Arg-Arg-Gly-Lys (RRGK) for CTSB (Figure 2A).[13] To increase the stability of the peptide in serum and cells, they modified the peptide at the C-terminal with glycosylation, which can shield the peptide backbone and the C-terminal end from proteases.[14] They found that the stability and glycosylation of the molecules play a crucial role in the final biological activity. Cell experiments suggested that CTSB-induced assembly of peptide precursors can induce LMP and activate the downstream cell death pathway of predominantly necroptotic apoptosis in glioblastoma cell death. Meanwhile, this strategy could enhance the activity of commercial chemotherapeutic agents by promoting drug escape from lysosomes.[13] This set of results reveals the potential for applying the lysosomal enzyme-triggered self-assembly strategy.

More recently, Liang et al. (Figure 2B) developed a Near-Infrared Photoacoustic (NIR PA) probe used for tumor-specific PA imaging, referred to as Cypate-CBT, which can self-assemble into Cypate-CBT-NPs by GSH reduction and CTSB cleavage and induces PA signal by aggregation, increased local concentration of the probe, and prolonged retention time, resulting in a more stable 4.7–4.9-fold enhancement of PA signal in CTSB-overexpressing cancer cells and tumors.[15] This study provides a novel strategy for enhancing PA signals to detect tumors with high sensitivity.

Another smart design of employing CTSB is reported by Ja-Hyoung group (Figure 3).[16] They utilized the Arg-Arg-Arg-Arg-Lys (RRRRK) sequence, a cell-penetrating peptide (CPP) sequence, and a CTSB substrate to achieve functional targeting of cancer lysosomes. By using the enzymatic cleavage function of CTSB, the peptide amphiphile is cleaved into more hydrophobic molecules in the lysosome and accumulates locally, which induces self-assembly to form long fibers, causing lysosomal swelling and membrane permeabilization. The authors found that the self-assembled nanostructures can cause the lysosomal disruption and induction of apoptosis in cancer cells with a very high selectivity index of up to 20. This work provides a new therapeutic method for drug-resistant cancers with a super-selective property. All these results demonstrated the promising future of using CTSB for inducing peptide self-assembly in the lysosome.[17]

Using Enzyme in Lysosome to Trigger Self-Assembly Indirectly

The indirect use of enzymes to trigger peptide self-assembly in the lysosome involves additional steps or components to mediate the self-assembly process. In this indirect approach, enzymes play a vital role, leading to the self-assembly of peptides or other molecules. This technique may be more complex than direct enzyme-triggered assembly but may offer the advantage of more carefully regulated and multifaceted control over the self-assembly process.[18]

Extracellular phosphatase, overexpressed in cancer cells, is another enzyme commonly involved in inducing peptide self-assembly in living cells[11b,19] and has received many achievements in constructing nanostructures in living systems. However, due to multiple phosphatases with similar functions inside and outside the cell, it is still challenging to utilize this strategy to form nanostructures in lysosomes to control cell behaviors precisely. Regarding this challenge, based on the acidic environment of the lysosomal lumen and the specific acid phosphatase

Table 1. Comparison of functional peptide self-assembly strategies in the lysosome.

Strategy	Pros	Cons
Using acidic pH of lysosome to trigger self-assembly	Universality: Almost all lysosomes have an acidic pH, so this is a broadly applicable strategy. Simplicity: It relies on a straightforward intrinsic property of lysosomes without needing external triggers. Predictability: The acidic environment of lysosomes is a well-characterized and consistent trigger for the design of pH-responsive materials.	Non-specificity: Acidic environment is a unique property of lysosomes in all types of cells, leading to potential off-target effects. Limited Control: The rate and extent of self-assembly might be hard to fine-tune since the pH can fluctuate and is not a singular value across all lysosomes. Potential for Premature Assembly: Peptides might aggregate in other mildly acidic environments before reaching lysosomes.
Using lysosomal enzyme to trigger self-assembly	Specificity: Many lysosomal enzymes have unique substrates, allowing for the design of peptides that only assemble when encountering these specific enzymes. Control: Enzyme-substrate interactions can be designed to provide more precise control over the timing and location of self-assembly. Functionality: Enzyme-triggered assemblies can be designed to release other therapeutic agents upon assembly, adding functionality to the system.	Complexity: Requires a more complex design to ensure specificity to lysosomal enzymes. Variability: Different cell types may have varying levels of lysosomal enzymes, affecting the consistency of the self-assembly process. Dependency on Enzyme Activity: The efficacy of this strategy is contingent upon the presence and activity level of enzymes, which can be affected by various factors such as enzyme inhibitors or the health state of the cell.
Using enzyme in lysosome to trigger self-assembly indirectly	Enhanced Control and Specificity: Combining pH and enzyme triggers can provide a two-step control mechanism that enhances the specificity to lysosomes. Synergy: Utilizing both triggers may yield a more robust system where self-assembly only occurs when both conditions are met, reducing the risk of off-target effects. Versatility: This approach can be tailored to respond to multiple environmental cues, potentially allowing for complex designs and applications.	Increased Complexity: The design of peptides that respond to two different stimuli is more complex and may require extensive optimization. Risk of Confounding Interactions: There is a potential for interactions between the two triggering mechanisms that may lead to unexpected results. Requirement for Precise Calibration: Achieving the correct balance between pH sensitivity and enzyme specificity requires precise calibration, which can be challenging.

(ACP), Wang and coworkers designed a multistage self-assembly strategy by introducing a "Trojan horse" protective motif,[20] achieving intracellular lysosome-specific self-assembly of the peptide (Figure 4). They introduced an O-[bis(dimethylamino)phosphono] tyrosine motif to avoid hydrolysis of the phosphotyrosine by phosphatases (e.g., alkaline phosphatase) on the cell membrane and cytoplasm, which can be dissociated when a precursor molecule enters the acidic lumen of the lysosome. Subsequently, with exposure to the enzymatic site, peptides self-assemble in response to ACP within the lysosome, realizing high efficiency and precise formation of nanostructures within the lysosome. As an example of an enzyme indirectly triggering the self-assembly of a peptide, this work demonstrates the potential of this multifactorial regulation regarding providing more precise control of the reaction.

Summary and Outlook

Lysosomal peptide self-assembly is an emerging field of nanomedicine, which endows the nanostructures formed on a spatiotemporal scale precisely to control cellular behavior. By harnessing the lysosome with the processes of cellular uptake, peptide precursors can self-assemble to form functional nanostructures inside the lysosome via different strategies (Table 1.) while locally increasing the concentration of therapeutics, thus improving the therapeutic index of precursors. Although the development of this field is in its infancy, it is a challenge and an opportunity to develop a new self-assembly system with the knowledge of understanding the biochemical properties of lysosomes. One essential requirement in this field is to explore the molecular space for self-assembly because most of the reported work is around the modification of serendipitously obtained precursors. The ideal system should be like the arrangement of functional proteins in cells, the current computational assisting design could be a strategy.[21] Another challenge is precisely controlling the formation of assemblies in the lysosome and fulfilling their function at the designed location. For example, most of the antigens need to be present on the cell surface for further recognition by immune cells. The current strategies used an acidic environment and CTSB in the lysosome. A better understanding of biological information in lysosomes could help us design efficient self-assembly systems for controlling cell behaviors.

Future work should pay more attention to characterizing the morphologies in living cells. Discovering the nanostructures formed by small molecules in cells is still challenging despite the atomistic structures in situ of assemblies. The rapid development of bio-electron microscopy (bio-EM) and fluorescent molecular labeling techniques in exploring protein structures could be a solution. These techniques allow researchers to directly visualize the nanostructures and their interactions with lysosomal membranes at high resolution and correlate the presence of peptide nanostructures with changes in lysosomal integrity, providing a mechanistic understanding of their role in LMP and other cell behaviors. Before observing the functions, how the monomers or oligomers of precursor interact with the cell membrane and are uptake into the cells to self-assemble into the higher-order structure is still a black box. A better

understanding of the structure-function relationship could be helpful in designing small molecules to target different processes of cells.

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Conflict of Interests

The authors declare no conflict of interest.

Keywords: self-assembly · peptide · lysosome · nanofibers · cancer therapy

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