JACS Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

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Perspective

Precise Regulations at the Subcellular Level through Intracellular Polymerization, Assembly, and Transformation

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Cite This: *JACS Au* 2024, 4, 4162−4186
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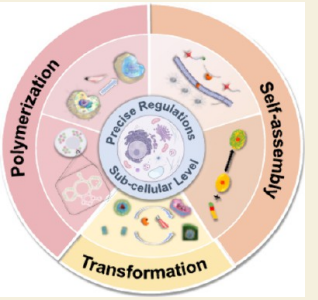
ABSTRACT: A living cell is an intricate machine that creates subregions to operate cell functions effectively. Subcellular dysfunction has been identified as a potential druggable target for successful drug design and therapy. The treatments based on intracellular polymerization, self-assembly, or transformation offer various advantages, including enhanced blood circulation of monomers, long-term drug delivery pharmacokinetics, low drug resistance, and the ability to target deep tissues and organelles. In this review, we discuss the latest developments of intracellular synthesis applied to precisely control cellular functions. First, we discuss the design and applications of endogenous and exogenous stimuli-triggered intracellular polymerization, self-assembly, and dynamic morphology transformation of biomolecules at the subcellular level. Second, we highlight the benefits of these strategies applied in cancer diagnosis and treatment and modulating cellular states or cell metabolism of living systems. Finally, we conclude the recent progress in this field, discuss future perspectives, analyze the challenges of the intracellular functional reactions for regulation, and find future opportunities.



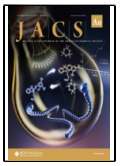
KEYWORDS: *intracellular polymerization, intracellular self-assembly, endogenous stimulus-triggering, exogenous stimulus-triggering, subcellular manipulation, bioactive materials*

1. INTRODUCTION

Various complex biological reactions involved in catabolic and anabolic processes within living cells produce biomolecules to regulate cellular functions.[1] Subcellular compartments including organelles as naturally occurring "nanoreactors" provide required microenvironments for a series of biological reactions, such as specific pH,[2] redox environment,[3] molecular crowding, or the presence of certain enzymes.[4,5] For instance, mitochondria are responsible for cellular aerobic respiration and energy production, where the production of ATP and NADH related energy-releasing reactions are initiated and regulated here.[6,7] The Golgi apparatus, as a secretory machine, is not only involved in cellular endocytosis and exocytosis, but also associated with the formation of lysosomes.[8−10] However, the disorder of organelles or abnormal changes in microenvironment components can interfere with cell function, resulting in various diseases.[4,11,12] Therefore, it is essential to monitor and regulate intracellular biological processes at the subcellular level. Traditional small molecule therapeutics face several intrinsic limitations, including low cancer-targeting capability,[13] poor solubility,[14] short drug retention time,[15,16] low selectivity or bioavailability,[17,18] and possible drug resistance.[19−21] Alternatively, targeting multiple subcellular compartments at different locations within cells could lead to potential effective pharmaceuticals.[22,23] Thus, it is essential to design materials for precise regulation of biological process at the subcellular level.

In recent years, significant efforts have been devoted to the intracellular synthesis of functional polymers, which introduce non-natural polymers into cells to regulate cellular functions.[24−26] In contrast to wet-chemical synthesis, intracellular synthesis can form large aggregates, facilitating their accumulation at targeting sites to improve the physiological stability with prolonged half-life.[27,28] This emerging approach has been applied in various biomedical research areas, from regulating cell behavior to targeted therapeutic interventions, and holds great potential for overcoming existing challenges in traditional therapies.[24,26,29] Besides polymer synthesis, self-assembly of polymers, a process in which monomers form supramolecular structures via noncovalent interactions, has emerged as a vital strategy for constructing functional biomaterials.[27,30] Intracellular self-assembly is achieved through generated monomeric biomolecules within cell aggregated together in a well ordered pattern to form complex materials,[31−33] with enhanced therapeutic effects and reduced drug efflux. In addition, nanoparticles that undergo morphological transformation in living cells could interact with subcellular components and modulate functions at the subcellular

Received: September 15, 2024
Revised: October 18, 2024
Accepted: October 22, 2024
Published: November 1, 2024



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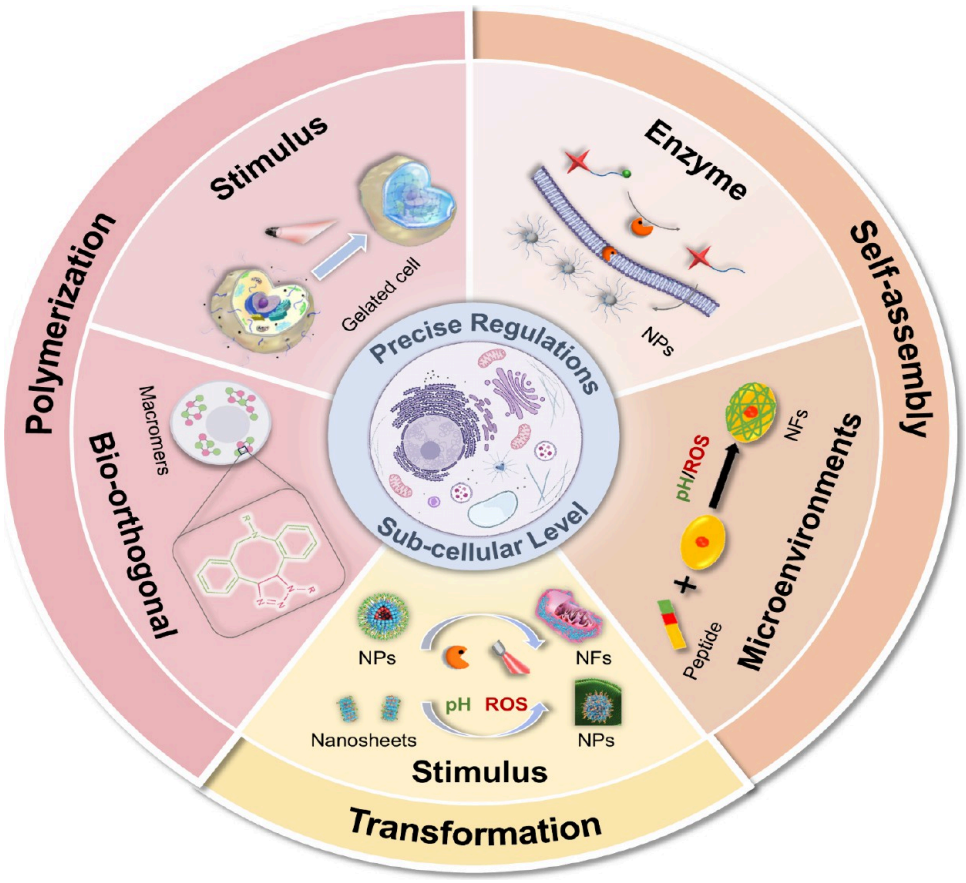
Scheme 1. Overview of Intracellular Polymerization, Self-Assembly, or Transformation-Based Strategies for Effective Regulation of Biological Processes at the Subcellular Level



level.[34−37] Recent studies have shown that autocatalytic morphology transformations could enhance drug accumulation.[38] Overall, in this review we summarize the recent progress and applications of functional intracellular polymerization, self-assembly, and morphological transformations. The following areas are highlighted: (1) Bio-orthogonal polymerization, which are chemical reactions occurring within biological organisms without interfering with other biochemical processes;[39,40] (2) light- or reactive oxygen species (ROS)-triggered intracellular polymerization, which enables precise control of drug release or other biological effects at designated time and location;[28,41] (3) intracellular self-assembly triggered by corresponding intracellular microenvironments; (4) enzymes-meditated self-assembly; and (5) morphological transformation of peptide assemblies in response to cellular environments or external stimuli (Scheme 1). Finally, we conclude the advantages of these techniques in achieving precise control at the subcellular level and their potential applications in nanomedicine.

2. RECENT PROGRESS IN INTRACELLULAR POLYMERIZATION, ASSEMBLY, AND TRANSFORMATION

The regulation of cellular activities by polymers has been widely applied in cell biology, including DNA delivery carriers and drug probes for cell sensing and materials that mimic biological functions.[42−45] However, these polymeric structures are usually synthesized at the lab bench and then introduced into living organisms. Currently, certain intracellular reactions have been utilized to synthesize a series of materials for nanomedicine.[28,30,46] Endogenous stimuli have been employed to regulate polymerization or self-assembly of polymers within cells (e.g., enzyme,[47] pH,[48] and ROS[49]). Exogenous stimuli, such as light[41] and magnetic fields,[50] can remotely target specific sites and initiate desired intracellular synthesis or self-assembly. This section outlines and summarizes recent developments of intracellular polymerization, assembly, and transformation.

2.1. Intracellular Polymerization

Polymerization is the process by which small molecules (monomers) covalently join and form into large molecules (polymers) through chemical reactions.[24,26,29] Life-sustaining biomacromolecules, including RNA, DNA, polysaccharides, and proteins, are synthesized within cells through the process of polymerizing smaller biomolecules, which is essential for basic life process and cell proliferation.[30,46] Therefore, intracellular polymerization presents a significant opportunity to merge synthetic chemistry with biology, enabling the construction of bioactive polymer or materials within living organisms.[28] Various types of chemical reactions have been applied in polymerization in living cells, such as amino-yne reaction, Diels−Alder reaction, Cu(I)-catalyzed radical polymerization, light induced radical polymerization and hyperbranched polymerization.[28]

2.1.1. Bio-orthogonal Intracellular Polymerization. In 2022, the Nobel Prize in Chemistry was awarded to Carolyn Bertozzi, Morten Meldal, and K. Barry Sharpless for their pioneering work in click chemistry and bio-orthogonal

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JACS Au 2024, 4, 4162−4186

chemistry, which involve reactions that occur in living systems without interfering with native biochemical processes.[39,40] Such an *in vivo* manipulation of biomolecules holds significant potential for advancing precise and efficient therapeutic methods against a series of diseases.

For instance, the classic spontaneous amino-yne click polymerization is characterized as a high reactivity and selectivity reaction without the need of a catalyst, which has been widely utilized as a bio-orthogonal reaction.[51,52] Tang and co-workers developed a "lab-in-cell" concept of an aggregation-induced emission (AIE)-active polymer based on the amino-yne click reaction.[53] Specifically, poly(β-amino-acrylate) was spontaneously polymerized by tetraphenylethene-containing diamine (amine monomer) and the carbonyl group activated terminal diyne (yne monomer) in HeLa cells. Due to the restriction of the intramolecular motion of tetraphenylethene, this polymer could also simultaneously illuminate the cells. Furthermore, the AIE-active polymer could selectively disturb the structures of tubulin and actin, demonstrating significant potential for antitumor applications. This intracellular polymerization provides an alternative approach for integrating click polymerization into diagnostic and therapeutic strategies. In addition, tumor metastasis is the leading cause of death in patients with malignant tumors, and immune-cell-based cancer therapy holds great potential to address this challenge.[54,55] However, its widespread use is hindered by complex preparation processes, insufficient targeting, and limited controllability.[56,57] Traditional bio-orthogonal chemistry can also be used to regulate the *in vivo* biological activity of immune cells. Previous reports showed that photoinitiated click chemistry could be used to enhance immunotherapeutic efficiency.[58] Core−shell upconversion nanoparticles with multiple dibenzocyclooctyne (DBCO) groups could attach to long single-stranded DNA, and then form a cross-linked network with natural killer (NK-92) cells, resulting in a high level of tumor cell inhibition. Photosensitive chemical linker DNA was used to shrink the DBCO−DNA chains and shield the DBCO groups. Upon light activation, the DNA scaffolds formed a cross-linked network through a photoinitiated reaction, which inhibited tumor cell migration by extending the DNA chains from the surface of nanoparticles. Additionally, efficient cellular assembly enhanced the communication between tumor cells and NK-92 cells, promoting NK-92 cells to exert strong immune functions by secreting large quantities of cytokines (perforin, granzyme B, TNF-α, and IFN-γ),[59,60] ultimately improving the efficacy of metastasis suppression.

RNA interference (RNAi) techniques are widely utilized in therapeutics applications.[61] Previously developed siRNA delivery systems relied on environmental factors to activate the high-efficiency payload of siRNA.[62−64] These factors include the variations in extracellular an intracellular redox potential, ATP concentration, and pH gradients.[62−64] However, dynamic endogenous environments are difficult to control, leading to premature siRNA activation and off-target. To address this challenge, Royzen and co-workers reported an siRNA delivery system based on bio-orthogonal chemistry.[65] They engineered superparamagnetic iron oxide nanoparticles (NPs) coated with biodegradable and biocompatible dextran. As shown in Figure 1, the inverse electron-demanded Diels−Alder (IEDDA) reaction between trans-cyclooctene (TCO) and tetrazine was used to trigger siRNA activation. The NP-TCO-siRNA construct ensures that the siRNA payload is

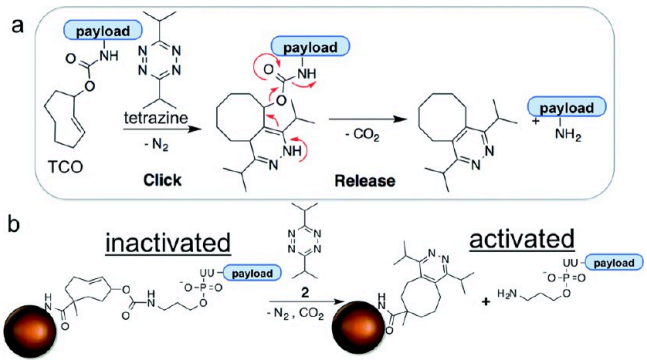

Figure 1. Schematic illustration of the siRNA activation triggered by bio-orthogonal chemistry. (a) IEDDA reaction between TCO and tetrazine. (b) Immobilized siRNA payload activated process during tetrazine introduction. Reproduced with permission from ref 65. Copyright 2017, Royal Society of Chemistry.

anchored to the NP surface, preventing it from prematurely interacting with the RNA interference specificity complex. SiRNA release is triggered only upon the addition of tetrazine, which is a bio-orthogonal trigger and initiates a bond-cleaving cascade, freeing the siRNA from the nanoparticle surface. Once activated, the siRNA targets the CDK8 gene, silencing the oncogene and inhibiting the proliferation of breast cancer cells.

Certain animals enter a reversible protective stasis by vitrifying their cytosol with polymeric molecules, such as proteins and polysaccharides, to survive extreme conditions.[66] The cytosol, already a crowded environment containing various organelles, vesicles, and cytoskeletal networks, experiences additional intracellular crowding during gelation. This increased density reduces diffusion-dependent reaction rates and impairs numerous biochemical processes.[66] Anseth and co-workers utilized click chemistry to create intracellular polymers that induce reversible molecular stasis.[40] As illustrated in Figure 2, complementary poly(ethylene glycol) macromers were transfected into mammalian cells using sequential lipofectamine, followed by intracellular cross-linking through bio-orthogonal strain-promoted azide−alkyne cycloaddition click reactions. This cross-linking led to various types of cellular dysfunction, including decreased DNA replication, inefficient protein synthesis, upregulated quiescent population, and disordered cytosol viscosity and cytoskeletal structure. However, upon exposure to light, the cross-linking could be reversed by incorporating photodegradable nitrobenzyl moieties into the polymer backbone, allowing the cell to return to its normal state. This approach of reversible intracellular cross-linking though bio-orthogonal reactions offers a promising platform for modulating essential cellular processes and functions.

2.1.2. Stimulus-Triggered Intracellular Polymerization. Non-natural intracellular polymerization including intracellular biological orthogonal reactions enables the introduction of multiple mechanisms for controlling cellular function.[26,67] Biomacromolecules are crucial for regulating biological processes, but they can interfere or even quench intracellular non-natural polymerization reactions due to competitive inhibition or inactivation.[68] Therefore, various endogenous and exogenous stimuli were selected and set as triggers to initiate intracellular polymerization for avoiding insufficient efficiency.[28,41]

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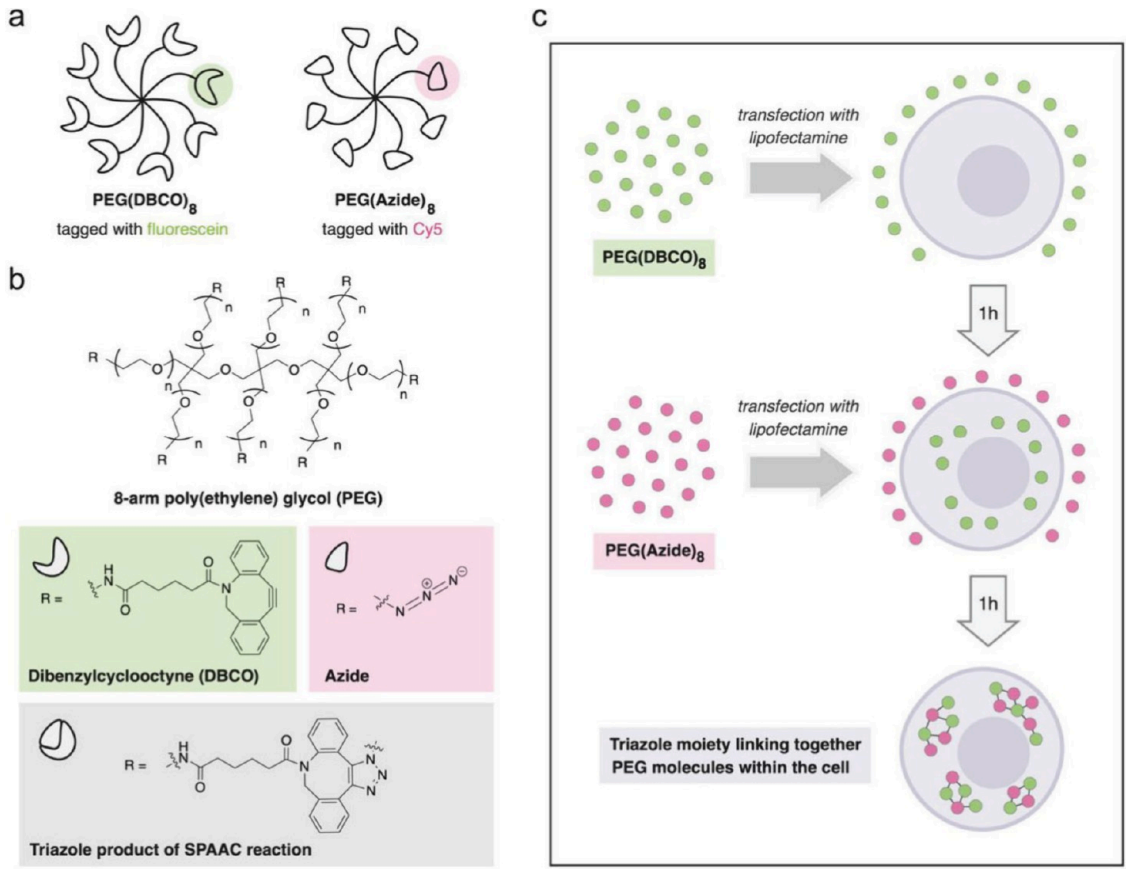

Figure 2. Schematic illustration of SPAAC cross-linking chemistry applications. (a) Diagram of strain-promoted azide−alkyne cycloaddition (SPAAC)-functionalized PEG macromers. (b) Structure of 8-arm PEG with R various groups. (c) Schematic representation of PEG macromers being transfected into cells via lipofectamine. Reproduced with permission from ref 40. Copyright 2022, Wiley-VCH.

Cu(I) complex-catalyzed radical polymerization can be performed under complex physiological conditions.[69,70] However, uncontrolled local accumulation of Cu(I) catalysts and cytotoxicity caused by Cu(I) agents and their ligands pose significant challenges in balancing the catalytic activity and cell viability.[71,72] To address this issue, Wang and co-workers developed an intracellular free radical polymerization system regulated by redox reactions in the tumor microenvironment.[73] As illustrated in Figure 3, histidine was chosen as a ligand to prepare the Cu-His complexes. In the presence of endogenous glutathione (GSH) and sodium ascorbate (NaAsc), a low dose of the Cu(II)−histidine complex was continuously reduced into an active Cu(I) catalyst inside living cells. The active Cu(I) then initiated the polymerization of *N*-hydroxyethyl acrylamide, with the molecular weight and conversion of the polymer increasing over time. The acryloyl paclitaxel was polymerized into a paclitaxel-bearing polymer (poly-PTX) by the active Cu(I). These polymers demonstrated superior cell-labeling capabilities and greater cell apoptosis induction compared to the monomeric forms of fluorescent and drug molecules. Additionally, poly-PTX exhibited negligible cytotoxicity to normal cells, thereby minimizing chemotherapy side effects in this treatment system.

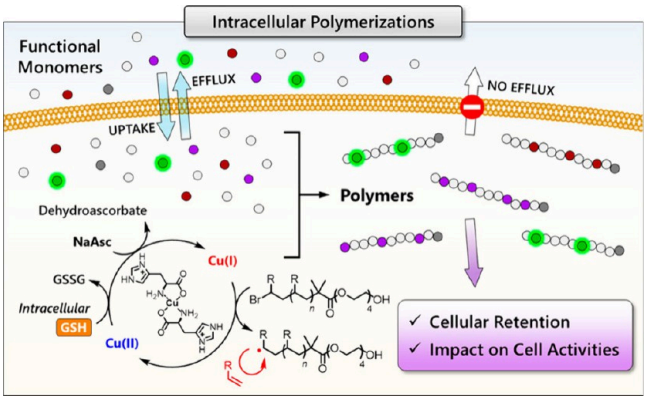

Figure 3. Schematic illustration of Cu(I)-catalyzed radical intracellular polymerization. Monomers and initiator are taken up by the living cells, and extracellular sodium ascorbate (NaAsc) is added to the cell culture. In the hypoxic tumor microenvironment, Cu(II) is reduced to Cu(I) by NaAsc and intracellular GSH, activating the dormant alkyl halide initiator and initiating polymerization. Reproduced with permission from ref 73. Copyright 2021, American Chemical Society.

Light, as an exogenous stimulus, provides energy to initiate photoreactions without interfering with most biomolecules present within cells.[30] Despite its potential, radical polymerization has rarely been applied in living organisms to modulate cellular functions or track cells. Bradley and co-workers developed a photopolymerization method to generate polymers within cells.[74] As shown in Figure 4, they introduced a light-mediated free-radical polymerization technique using a biocompatible initiator and a range of monomers.[74] 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylphenylacetone (Irgacure 2959), a biocompatible photosensitizer, was selected to initiate the radical polymerization of vinyl-containing com-

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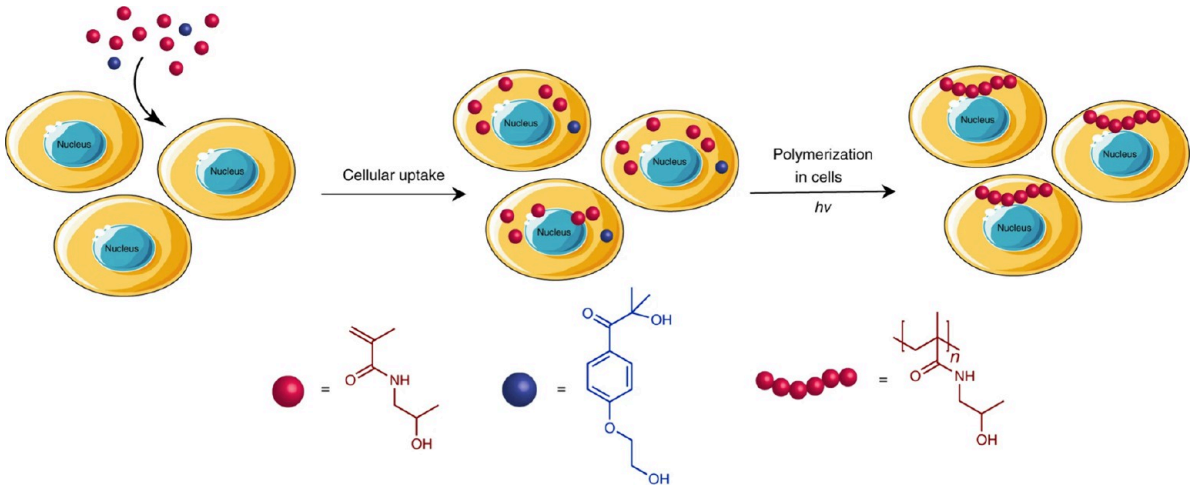

Figure 4. Schematic illustration of manipulating and tracking cellular behavior through radical polymerization within living cells. The monomer HPMA and initiator Irgacure 2959 were employed to initiate photopolymerization upon illumination at 365 nm. Reproduced with permission.[74] Copyright 2019, Nature Publishing Group.

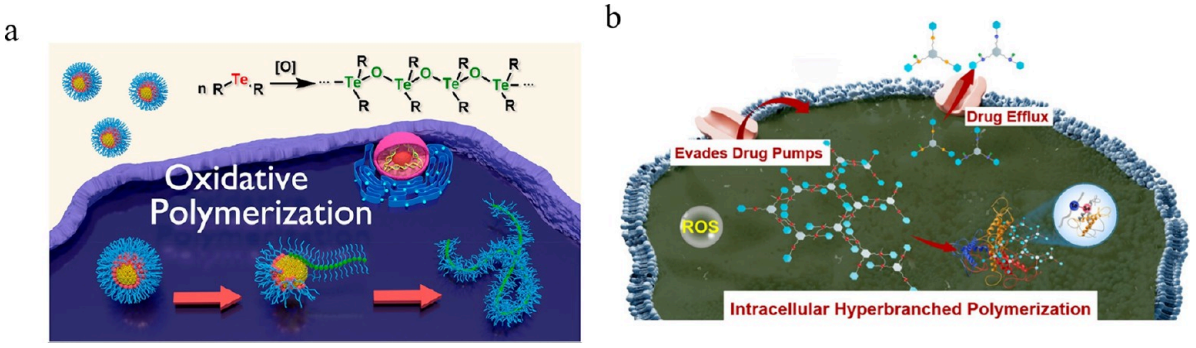

Figure 5. Schematic illustration of ROS triggered polymerization. (a) Oxidation polymerization utilized on Te nanoreservoirs (TNRs). Reproduced with permission from ref 49. Copyright 2021, American Chemical Society. (b) Hyperbranched polymerization utilizing B3−Te. Reproduced with permission from ref 82. Copyright 2023, American Chemical Society.

pounds through ultraviolet irradiation. Several compounds, such as *N*-hydroxypropylacrylamide and sodium p-vinylbenzenesulfate, were used as monomers. The results demonstrated that free-radical photopolymerization can be performed inside HeLa cells incubated with various monomers. This method was shown to regulate cell migration efficiency and achieve stable fluorescent labeling of cells for live-cell tracking, offering broad utility in regenerative medicine and adoptive cell therapy for monitoring cellular distribution and survival.

In addition to modifying cellular processes in living systems, intracellular polymerization has been utilized to create inanimate, cell-like biomaterials for various biomedical applications. Lee and co-workers reported a cellular fixation method mediated by the intracellular assembly of hydrogel monomers.[75] Unlike conventional fixation techniques that relied on chemical cross-linking, this approach employs intracellular photoactivated radical polymerization of PEG diacrylate (PEG-DA) to induce hydrogel-mediated solidification of the cytosol without disrupting the cell membrane integrity. Hydrogel monomers and photoinitiators were introduced into the intracellular domain of cells, following transient membrane poration. By the formation of a hydrogel network, the cell membrane is stabilized, preserving cellular features with enhanced structural robustness. This system holds significant potential for applications in membrane research and biomaterials engineering.

Geng and co-workers developed a method for tumor precision therapy using light-controlled intracellular polymerization within a prodrug system, which was designed to reduce side effects and enhance treatment efficacy in cancer therapies.[76] This photoactivatable approach was based on intracellular photoinduced electron transfer-reversible addition−fragmentation chain-transfer polymerization. The regularity of the polymers was significantly improved by narrowing the molecular weight distribution, which enhanced the spatiotemporal control and repeatability of the intracellular polymerization system. Through cytotoxicity screening, they identified a class of acrylamide monomers with low inherent toxicity that, upon intracellular polymerization, exhibit increased cytotoxicity, inducing apoptosis and necrosis in tumor cells.

ROS, including hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$), and hydroxyl radical ($OH^{\cdot}$),[77] play an important role as secondary messengers in cellular processes such as cell growth, apoptosis, and migration.[77−79] However, excessive ROS production can cause oxidative stress, leading to damage to DNA, proteins, and the lipid bilayer of the cell plasma.[78] Certain tellurium (Te)-containing-containing molecules exhibit instant and ultrasensitive responsiveness to ROS, even at concentrations as low as 100 μM.[80,81] In 2021, Xu and co-

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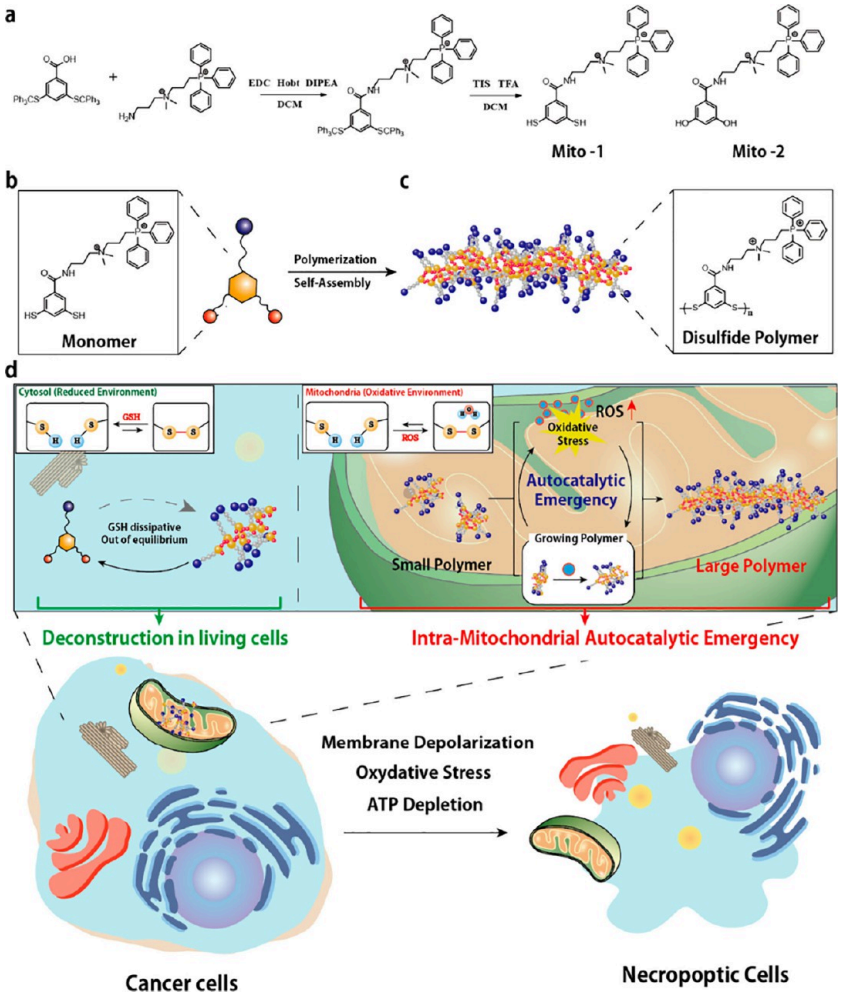

Figure 6. Schematic illustration of mitochondrial polymerization-induced self-assembly (Mito-PISA). (a) Chemical structure of the monomer. (b, c) Assembly process of a disulfide-based polymer. (d) Mito-PISA triggered mitochondrial dysfunction and induced necroptosis in cancer cells. Reproduced with permission from ref 92. Copyright 2021, American Chemical Society.

workers reported an oxidative intracellular polymerization triggered by intracellular ROS (Figure 5a).[49] The polymerization degree of Te nanoreservoirs increased with increasing H_2O_2 concentration. This ROS-driven polymerization occurred selectively in cancer cells, where ROS levels are elevated, but not in normal cells with basal ROS levels. These polymerization processes ultimately led to ROS-induced damage and cancer cell death.

Hyperbranched polymers offer several advantages, such as a larger surface area for targeting and multistage branched structures to overcoming drug efflux mechanisms.[83−85] Xu and co-workers reported intracellular hyperbranched polymerization based on the oxidative polymerization of organtellurides in a redox environment.[82] As shown in Figure 5b, an ROS-responsive eliminable compound, the B3−Te monomer, containing three tellurium atoms, was synthesized for oxidative polymerization. Due to the reversible redox properties of organotellurium, B3−Te exhibited reversible reactivity to ROS and underwent hyperbranched polymerization within living cells. The resulting Te−O-containing polymeric products disrupted the cellular antioxidant system by interacting with Te(+4) and selenoproteins, which are critical to cellular antioxidant defense. The resulting Te−O-containing polymeric products disrupted the cellular antioxidant system by interacting with Te(+4) and selenoproteins, which are critical to the cellular antioxidant defense. This disruption selectively induced apoptosis of cancer cells. Additionally, the polymers formed branched-chain nanostructures that could evade the drug efflux pump, ensuring that the hyperbranched polymerization provided long-lasting therapeutic effects. *In vivo* studies demonstrated that organotelluride-mediated hyperbranched polymerization achieved selective anticancer effects with minimal side effects on normal tissues, offering a promising approach for targeted cancer therapy.

2.2. Intracellular Self-Assembly

Self-assembly is the phenomenon where a system of existing components spontaneously organizes itself from into a disordered state an organized structure or pattern under appropriate conditions. Unlike polymerization, self-assembly primarily relies on noncovalent interactions, such as hydrogen bonds, van der Waals forces, and electrostatic interactions, and does not require direct external energy to initiate. This process arranges repeating units or active molecules on a nanometer or micron scale, aligning well with intracellular microenvironment, which typically exists at these dimensions.[30] Imbalance in the microenvironment can disrupt cellular function and lead to disease. Self-assembly of nonfunctional components into functional structures within living cells provides a promising

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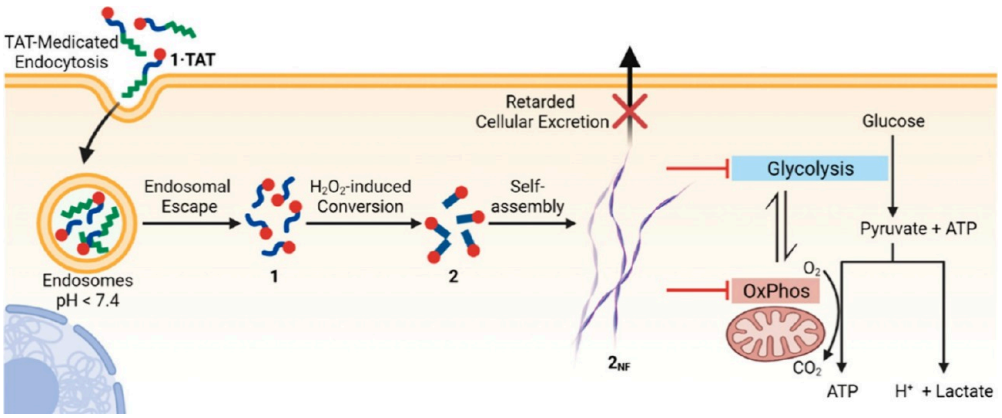

Figure 7. Illustrative schematic of the formation of linear platinum(II) complexes triggered by intracellular H_2O_2, subsequently self-assembled into nanofibers. These nanofibers impact the energy balance and interfere with normal cellular metabolic processes. Reproduced with permission from ref 93. Copyright 2022, American Chemical Society.

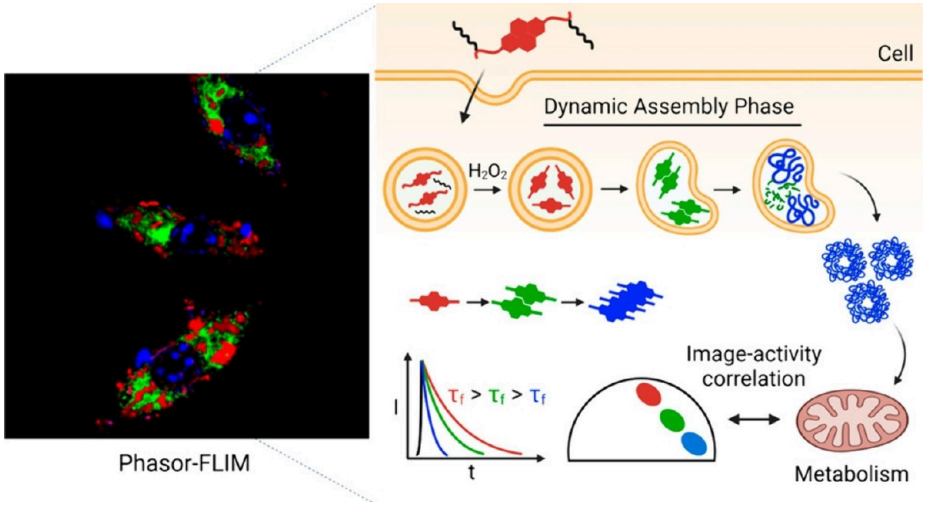

Figure 8. Schematic representation of the endosomal assembly of peptides. The assembly dynamics were tracked by phasor-fluorescence lifetime imaging.[94] Copyright 2024, American Chemical Society.

platform for precise control of subcellular processes. When designing chemical reactions in cells, factors such as the subcellular distribution of the reactants must be carefully considered.[86] Various internal stimuli can lower the energy barrier for self-assembly, facilitating these processes.[4,86] Intracellular self-assembling materials can be tailored to exploit differences between diseased and normal microenvironments, helping to minimize off-target effects during drug delivery, reduce toxicity, and enable precise regulation at the subcellular level.

2.2.1. ROS-Induced Self-Assembly. Mitochondria are essential for various cellular functions, including energy metabolism, mitochondrial biosynthesis, redox balance, and stress responses. They are also actively involved in tumorigenesis and proliferation.[87,88] ROS production is a critical step in the mitochondrial oxidative stress pathway and could be harnessed to induce intracellular self-assembly.[7] Polymerization-induced self-assembly (PISA) has emerged as a promising technique for the industrial-scale synthesis of polymeric materials used in drug delivery, medical imaging, and tissue engineering.[89] PISA could create synthetic nanostructures within cells that effectively interact with biomolecules,[90,91] offering potential for precise control over cellular functions and inspiring optimized therapeutic strategies. In 2021, Kim and co-workers reported a self-assembly system induced by intramitochondrial polymerization for regulating cell fate.[92] As shown in Figure 6, the monomer was synthesized by conjugating aromatic dithiol with quaternary ammonium-modified triphenylphosphine by 1-ethyl-3-(3(dimethylamino) propyl) carbodiimide coupling. Disulfide (−S−S−) cross-linking in polymers was facilitated by the high concentration of mitochondrial ROS (mtROS) present in cancer cell, though polymerization is less common in other parts of the cell with reducing environments. The polymerization of thiol-containing monomers further elevated mtROS levels, autocatalyzing the polymerization process and resulting in a fibrous polymer structure. The process ultimately involves mitochondrial dysfunction and necroptosis in cancer cells.

Specific metabolic pathways in mitochondria can also be used as targets to regulate metabolism by intracellular self-assembled materials. As shown in Figure 7, Weil and co-workers reported a platinum(II)-containing tripeptide, which consists of three parts: (1) a proassembling isopeptide caged by an boronic acid group; (2) a transporter peptide that could bind to the boronic acid; and (3) a platinum(II)-terpyridine complex attached to the alkynyl moiety on the amino terminus of the peptide.[93] Once released to the near-neutral (pH 7.4)

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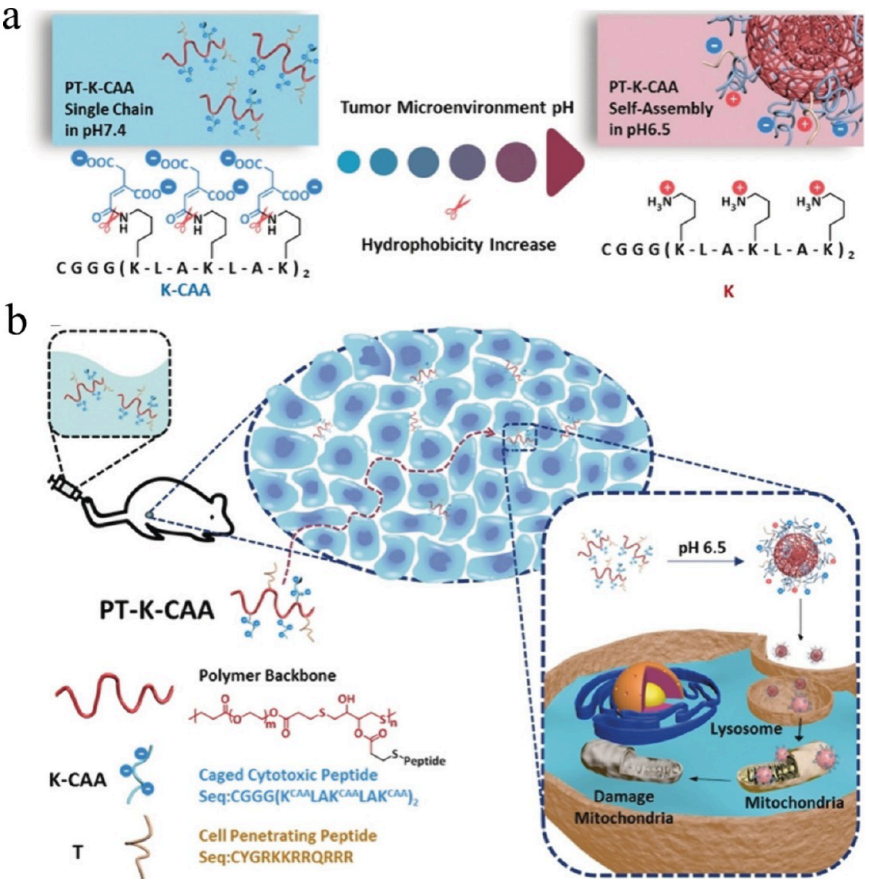

Figure 9. (a) Structure and self-assembly process of PT-K-CAA. (b) Schematic diagram illustrating the penetration and pH-responsive self-assembly of PT-K-CAA in the tumor microenvironment. Reproduced with permission from ref 86. Copyright 2019, Wiley-VCH.

cytosol, the endogenous H_2O_2 immolates the boronic acid cage, generating the serine residue with a primary amino group. Then the tripeptide could assemble into nanofibrillar upon intracellular molecular rearrangement. The resulting nanostructures could inhibit key metabolic processes, such as aerobic glycolysis and oxidative phosphorylation, and then effectively block ATP generation. This inhibition consequently led to a decreasing in ATP-dependent actin polymerization and a reduction in the activity of histone deacetylases.

Real-time visualization and monitoring of the process of intracellular polymerization and self-assembly are also critical for designing the active material to regulate cell fate. Ng and co-workers designed a proassembling peptide, and used imaging technologies and time-resolved extracellular flux analysis to capture intracellular molecular-level assembly processes.[94] As presented in Figure 8, the proassembling peptide could enter epithelial cells through endocytosis and transform into the supramolecular monomer upon exposure to endogenous H_2O_2. The confined environment of the endosome could guide the assembly of nanofibers, leading to the fusion and formation of distinct hollow clusters. Meanwhile, the real-time assembly dynamics were traced by phasor-fluorescence lifetime imaging. The biological response upon intracellular assembly analysis demonstrated that the initiation of assembly was crucial in disrupting endosomal function, significantly hindering the associated glucose-dependent metabolism and cellular respiration.

2.2.2. pH-Triggered Self-Assembly. The pH of normal cells is approximately 7.4, and cellular dysfunction can lead to pH alterations. Some studies have exploited abnormal pH levels to achieve pH-responsive drug release.[95−98] Additionally, amino acids, as fundamental building blocks of proteins, can be leveraged in molecularly designed peptide amphiphiles to mimic protein functions.[99] Various studies have demonstrated the potential of peptide amphiphiles and amino acid amphiphiles as drug carriers, pharmacologically active materials, cell scaffolds, and catalysts.[100] Maruyama and co-workers designed peptide amphiphiles that self-assemble into entangled nanofibers in response to small pH changes near neutral pH.[101] They synthesized a palmitoylated hexapeptide (C16-VVAEEE), a peptide amphiphile that formed entangled nanofibers in response to low intracellular pH (pHi). C16-VVAEEE exhibited cytotoxicity against cells with low pHi, and microscopy revealed the self-assembly of C16-VVAEEE within HEK293 cells. *In vivo* experiments demonstrated that the intracellular self-assembly of these peptide amphiphiles contributed significantly to selective cell death. This study suggests that the distinct pH microenvironment within living cells can serve as a trigger for intracellular self-assembly of peptide amphiphiles.

In cancer treatment, the therapeutic efficacy of nanomaterials is often limited by their inadequate penetration into solid tumors.[102,103] Wang and co-workers addressed this issue by developing polymer−peptide conjugates (PPCs) that generate KLAK-based nanofibers in mitochondria via acid-induced self-assembly.[86] As presented in Figure 9, a cytotoxic peptide, KLAK, was modified with cis-aconitic anhydride (CAA), a pH-sensitive moiety, and further coupled with a cell-penetrating

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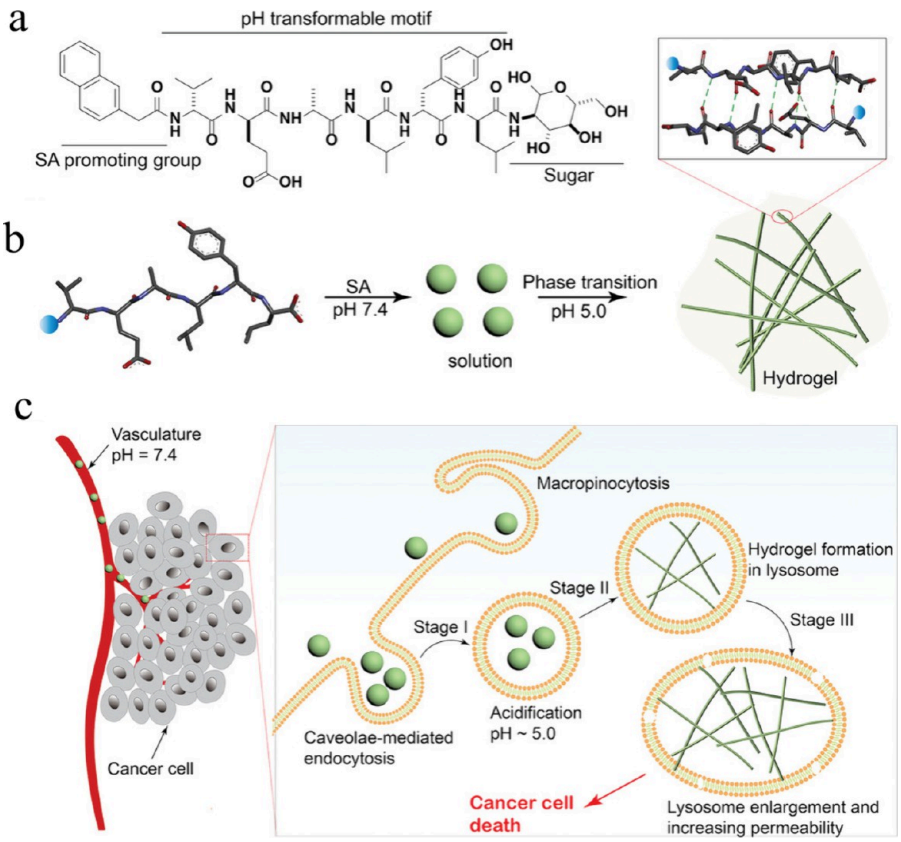

Figure 10. Schematic illustration of pH-responsive transformable peptides and their self-assembly process. (a) Typical molecular structure of LTP. (b) At pH 7.4, LTP self-assembled into nanoparticles, which then transformed into nanofibers under acidic conditions (pH 5.0). These nanofibers further entangled to form a self-supporting hydrogel. (c) Intracellular phase-transformation process of LTP. Stage I: LTP entered cancer cells via endocytosis. Stage II: LTP oligomers accumulated in lysosomes and transformed into nanofibers; Stage III: The nanofibers caused lysosome enlargement and led to cancer cell death. Reproduced with permission from ref 33. Copyright 2022, Wiley-VCH.

peptide, TAT, to a poly(β-thioester) backbone, resulting in the formation of PT-K-CAA. Due to its small size at pH 7.4, PT-K-CAA could penetrate deeply into solid tumors. Upon exposure to the acidic environment of tumors (pH 6.5), the CAA groups hydrolyze, prompting the self-assembly of PPCs into nanosized aggregates. Dynamic light scattering and transmission electron microscopy data revealed that particle size increased from 18 to 90 nm within 12 h at pH 6.5, with minimal change at pH 7.4. Fluorescence probe results indicated that the self-assembly process was driven by hydrophobicity. Under mildly acidic conditions, PT-K-CAA restored its α-helical structure and, consequently, its cytotoxicity. The PPC demonstrated no significant cytotoxicity under neutral conditions but was effective in killing tumor cells under acidic conditions. *In vivo*, CAA's responsiveness to the weak acidity of tumors facilitated the self-assembly of PPCs and the recovery of therapeutic activity. That *in situ* self-assembly strategy offers a promising approach for enhancing tumor permeability and improving the efficacy of nanomaterials in cancer therapy.

Recent evidence underscores the critical role of lysosomes in maintaining cellular homeostasis.[104] Changes or dysfunction in lysosome can significantly impact the development of various human diseases.[105] Lysosomes are notably acidic, with proton concentrations up to 1000 times higher than that in cytosol, making them key proton-storing organelles.[106] Targeting lysosomes presents promise for cancer treatment. The pH of lysosomes, typically ranging from 4.5 to 5.0, is significantly lower than that of cytosols.[95−98] Wang and co-workers developed a pH-responsive, lysosome targeting molecule (LTP) in living cells for cancer therapy.[33] As illustrated in Figure 10, the LTP designed incorporates several key components: (1) The Val−Glu−Ala−Leu−Tyr−Leu (VEALYL) segment, derived from human insulin protein, formed amyloid fibrils specifically under acidic conditions. (2) The N-terminal 2-naphthylacetyl modification enhances the self-assembly of peptides through aromatic−aromatic interactions. (3) C-Terminal glycosylation increases peptide stability against proteolytic degradation. At pH 7.4, LTP could form nanoparticles. Upon cellular uptake via endocytosis, LTP oligomers accumulated in lysosomes. At the acidic pH of 5.0, LTP underwent pH-sensitive transformation into nanofibers through noncovalent interaction, subsequently forming a self-supporting hydrogel. Lysosomal hydrogelation enlarged the lysosome within cancer cells and enhanced its permeability, leading to cancer cell death. In xenograft tumor models, lysosomal assemblies effectively mitigated drug resistance caused by lysosome sequestration.

Tang and co-workers developed Au(I)-disulfide nanosheets (NSs@TTVP), which involved an aggregation-induced emission photosensitizer.[107] The synthesis of nanosheets involved a two-stage procedure: (1) GSH was mixed with $HAuCl_4$ to produce Au nanoclusters; (2) introduction of cysteine led to the oxidation of all Au(0) atoms to Au(I), accompanied by the formation of disulfide bonds. Owing to the acidic sensitivity of cysteine residues, NSs@TTVP exhibited pH-responsive self-assembly capabilities, allowing

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for intracellular crystallization. In addition, the introduction of disulfide bonds into proteins promoted the formation of the Au(I)-disulfide complex, which has the potential to drive crystal growth within cells through crystallization-driven processes. As illustrated in Figure 11, NSs@TTVP remained

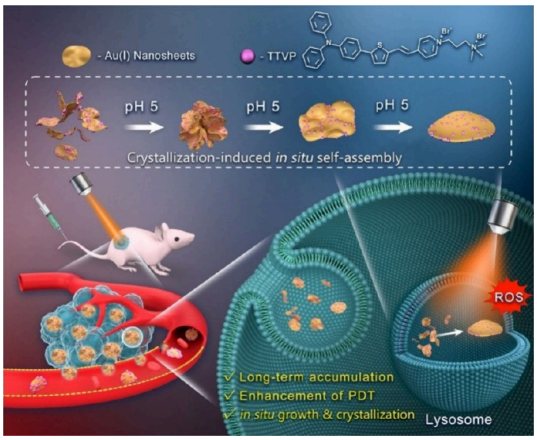

Figure 11. Schematic illustration of the acid-induced crystal self-assembly process of NSs@TTVP and its intracellular bioactivity. Reproduced with permission from ref 107. Copyright 2022, American Chemical Society.

stable as a single crystal at pH 7.4 but transformed into a polycrystalline structure at pH 5.0, with its diameter increasing from 80 nm to 1 μm during transformation. This reduction in the negative charge lessens the electrostatic repulsion between NSs, thereby promoting their fusion. In vivo experiments confirmed that this size increase due to self-assembly also occurred within the cells. Additionally, NSs@TTVP was employed for photodynamic therapy, generating ROS upon near-infrared (NIR) irradiation.

The cytoskeleton is essential for maintaining the morphology and function of eukaryotic cells and consists of three primary components: microtubules, microfilaments, and intermediate filaments.[108] Microtubules are crucial for processes such as cell metabolism, intracellular transport, and mitosis.[109] Small-molecule antimitotic agents, like paclitaxel and vinblastine have been used in cancer therapy due to their ability to bind to tubulin and alter microtubule dynamics during mitosis, thereby exhibiting potent anticancer effects.[110,111] However, these agents often cause significant neuropathy and drug resistance, which limit their clinical application. Zhang and co-workers developed a bioactive peptide using a molecular self-assembly strategy.[112] The peptide-based molecules were designed to adjust to pH and viscosity changes, during Golgi-endosome transport, thereby evading the endocytic pathway and interacting directly with microtubule arrays in a nonspecific manner. This interaction inhibited tubulin polymerization, induced pro-metaphase-metaphase oscillations, and then effectively suppressed cancer cell proliferation without causing notable neurotoxicity.

2.2.3. Enzyme Instituted Intracellular Self-Assembly.

Enzymes are crucial for cellular metabolism due to their high specificity and catalytic efficiency.[113,114] They play a pivotal role in all biochemical reactions within cells. In cancer cells, certain enzymes are often overexpressed or exhibit different activities compared to normal cells.[115−118] This variability in enzyme levels and activities can be exploited to target intracellular polymerization and self-assembly processes.

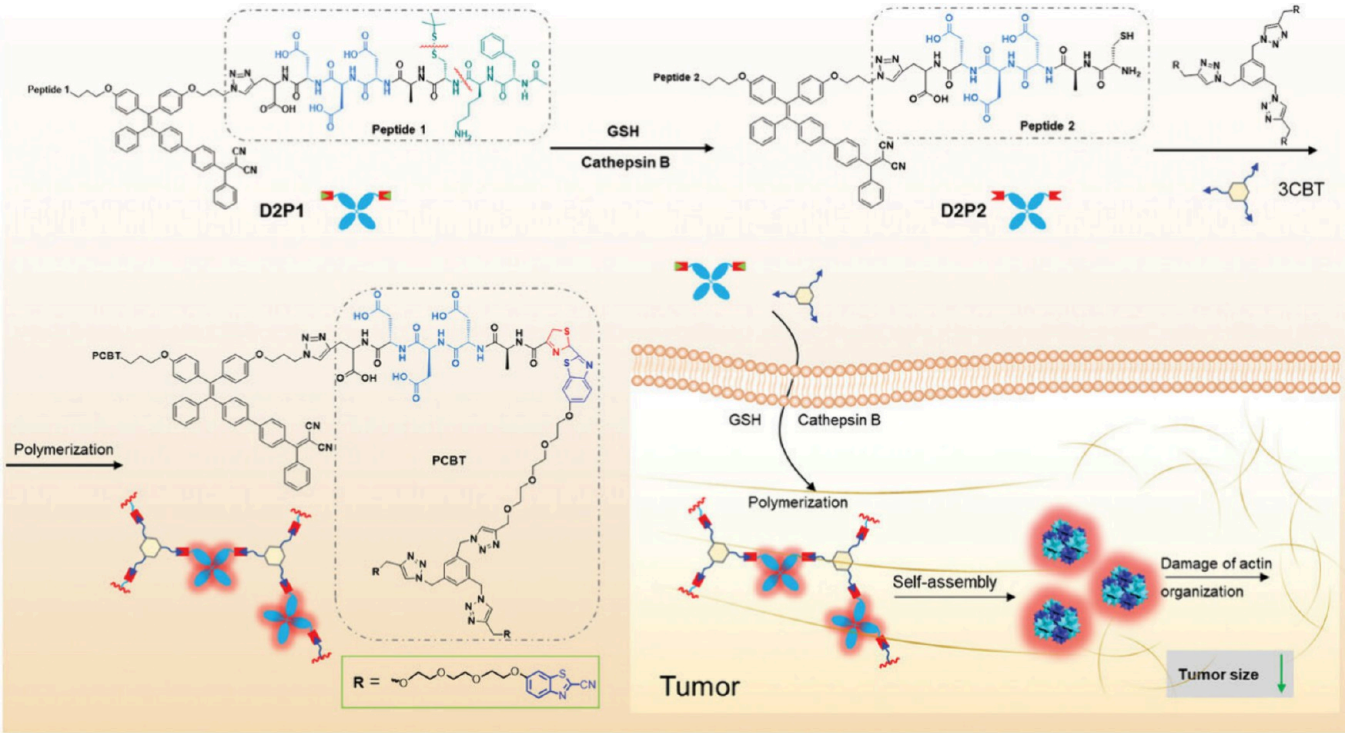

Figure 12. Schematic illustration of the synthesis route for enzyme-mediated intracellular reduction and condensation of D2P1 and 3CBT. This process results in the formation of AIEgen-based nanostructures that enhanced the fluorescence signal and improved tumor treatment efficacy. Reproduced with permission from ref 129. Copyright 2022, Wiley-VCH.

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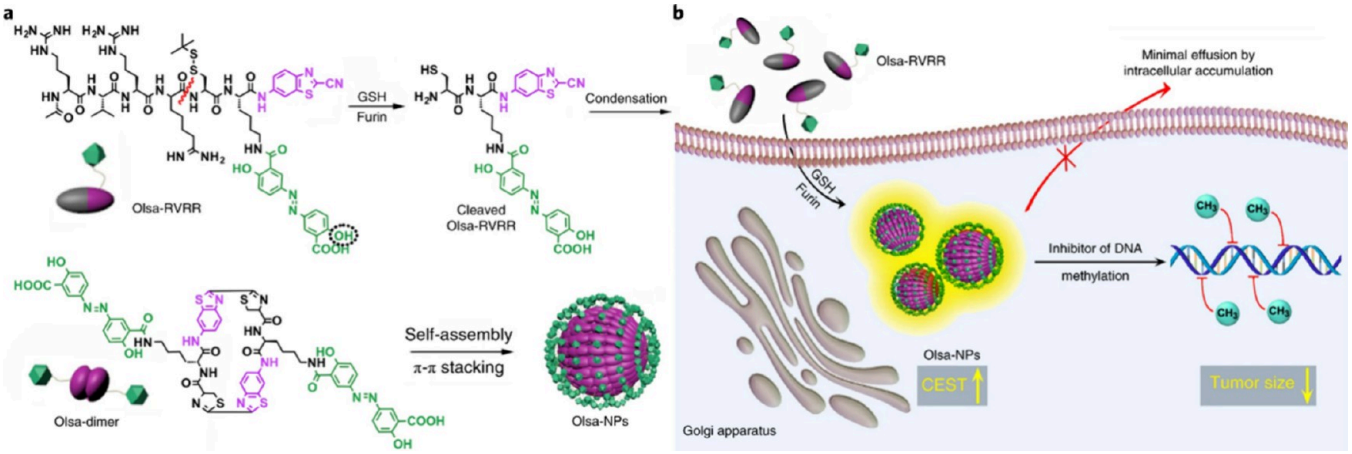

Figure 13. (a) Structure of Olsa-RVRR and the self-assembly mechanism of Olsa-NPs. (b) Illustration of the intracellular self-assembly of Olsa-RVRR triggered by furin and GSH, leading to the accumulation of Olsa-NPs within cells. This accumulation enhanced CEST imaging and inhibited DNA methylation for tumor therapy. Reproduced with permission from ref 133. Copyright 2019, Nature Publishing Group.

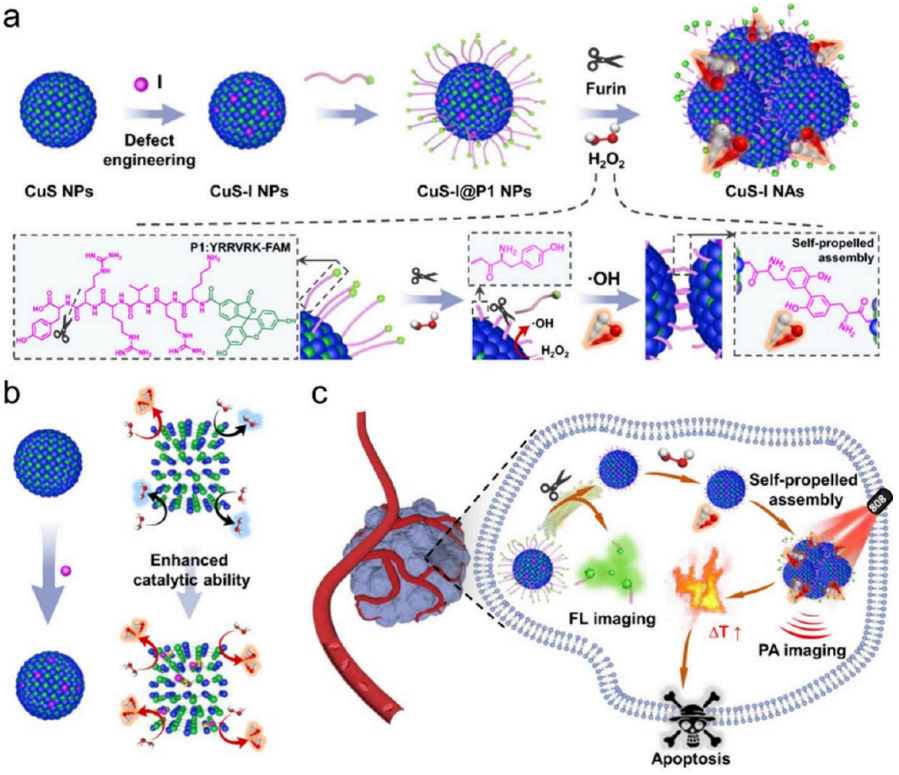

Figure 14. Schematic illustration of self-propelled assembly of CuS-I nanoassemblies (NAs). (a) Synthesis route of CuS-I@P1 NPs, which aggregated into CuS-I NAs through a hydroxyl self-catalytic reaction triggered by furin and H_2O_2. (b) Enhanced catalytic ability of CuS-I NPs for generating ·OH. (c) Specific activation of CuS-I NPs functionalities by overexpressed furin and H_2O_2, utilized for imaging and therapeutic applications. Reproduced with permission from ref 136. Copyright 2024, Nature Publishing Group.

Alkaline phosphatase (ALP) is a significant biomarkers of cancer diagnosis due to its abnormal expression in various cancers,[119,120] including breast,[121] prostate,[122] and kidney cancers.[123] Liang and co-workers developed an ALP-triggered self-assembly near-infrared photoacoustic probe for tumor imaging.[124] Ir775-phe-phe-tyr (H_2PO_3)−OH integrated the near-infrared dye IR775, which served as both a contrast agent and a hydrophobic agent. In the tumor microenvironment, ALP dephosphorylated Ir775-phe-phe-tyr (H_2PO_3)−OH, converting it into IR775-Phe-Phe-Tyr-OH, which exhibited a strong hydrophobic effect. This modification facilitated the uptake of the probe by tumor cells, leading to self-assembly into nanoparticles. The resultant nanoparticles quenched near-infrared fluorescence while enhancing photoacoustic signals, thereby improving photoacoustic imaging.

The 5-year survival rate of cancer patients is significantly higher when diagnosed at early stages compared to late stages, highlighting the importance of early diagnosis in cancer therapy.[19,125] Cathepsin B, a lysosomal cysteine protease, plays a key role in protein and organelle degradation, antigen presentation, and the execution of cell death pathways.[126] Abnormal cathepsin B activity is linked to various cancers,

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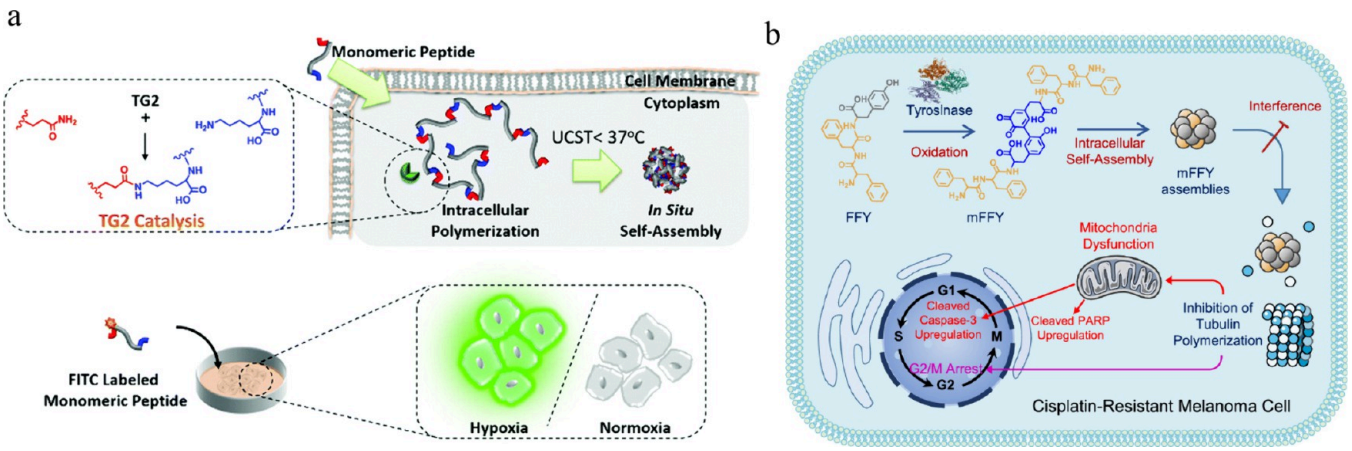

Figure 15. (a) Schematic illustration of intracellular polymerization specifically catalyzed by TG2, by temperature-induced self-assembly, designed for imaging hypoxic neuroblastoma. Reproduced with permission from ref 140. Copyright 2019, Royal Society of Chemistry. (b) Schematic illustration of tyrosinase-induced tripeptide assemblies and their intrinsic biological function in reversing drug resistance by inducing apoptosis in melanoma cells. Reproduced with permission from ref 145. Copyright 2022, American Chemical Society.

including colorectal, prostate, ovarian, and breast cancers.[127] Recently, AIEgen-based photosensitizers have shown the ability to emit strong fluorescence and generate large amounts of ROS in their aggregated state.[128] As shown in Figure 12, Liu and co-workers developed a tumor-specific intracellular polymerization technique using cathepsin B-mediated AIEgen for both imaging and inhibiting tumor growth.[129] They designed a pair of molecules containing AIEgen and a side-protected cysteine as the response unit. These molecules can be reduced by GSH and cleaved by Cathepsin B simultaneously. Once the AIEgen-peptide conjugate and cyanobenzothiazole entered the tumor cells, the CBT-cysteine condensation reaction occurred, forming nanoaggregates within tumor cells. These nanoaggregates enhanced fluorescent signals, disrupted actin organization, and subsequently controlled cellular motility, leading to improved tumor treatment efficacy.

Furin is a trans-Golgi protein convertase that is upregulated in various malignancies, including squamous cell carcinoma and glioblastoma.[130] It cleaves peptides at specific sites (Arg-X-Lys/Arg-Arg-X), which can be leveraged for targeted therapies.[131] Olsalazine (Olsa), known for its broad-spectrum anticancer properties and use as a chemical exchange saturation transfer (CEST) magnetic resonance imaging (MRI) contrast agent.[132] Bulte and co-workers developed a furin-mediated intracellular self-assembly strategy to improve the MRI signal and enhance anticancer efficacy.[133] Their approach, detailed in Figure 13, involved the use of Olsa-RVRR, a compound consisting of furin enzyme substrates (RVRR), Olsa, and 2-cyano benzothiazole. Upon entering cells that overexpress furin, Olsa-RVRR underwent cleavage by furin and reduction by GSH. These products then self-assembled into nanoparticles (Olsa-NPs) via $\pi-\pi$ stacking interactions between Olsa dimers. This self-assembly prolonged the exposure time of Olsa, enhanced the CEST MRI signal, and improved the DNA methylation inhibition effect of Olsa. This furin-targeted platform holds promise for improved tumor imaging, drug accumulation, and therapeutic response monitoring.

Dimerization of tyrosine (Tyr) is a natural and biocompatible free radical reaction without that does not require synthetic functional groups, making it suitable for *in vivo* applications.[134] Dityrosine cross-linking had been widely used in cell imaging, material synthesis, and photocatalysis due to its ability to form stable structures.[135] Ling and co-workers utilized this property for manipulating intracellular nanoparticle assembly.[136] Their approach, depicted in Figure 14, involved a Tyr peptide (P1) that is responsive to furin, which was introduced onto the surface of the CuS-I NPs as a ligand. The CuS-I@P1 NPs were modified with Tyr, which underwent furin-guided condensation reactions to form CuS-I nanoparticle assemblies (CuS-I NAs). This method allows for targeted and controllable assembly, enhancing photothermal effects and photoacoustic and fluorescence imaging. Additionally, the imaging capability of CuS-I@P1 NPs could be triggered by overexpressed H_2O_2 in tumor cells.

Transglutaminase 2 plays a significant role in the pathogenesis of neurodegenerative diseases, and its expression is upregulated in response to hypoxia, which is a common feature of tumor lesions. TG2-catalyzed intracellular polymerization and *in situ* self-assembly strategies had been proposed for tumor imaging.[137−139] As illustrated in Figure 15a, Li and co-workers developed a hypoxic neuroblastoma cell imaging probe based on TG2-catalyzed polymerization and subsequent self-assembly of FITC-labeled elastin-like polypeptides.[140] They discovered that negatively charged amino acids enhanced the degree of polymerization, while hydrophobic amino acids increased the upper critical solution temperature (UCST), thereby facilitating the polymerization process. The results demonstrated that various elastin-like polypeptides exhibited UCST behavior. This TG2-catalyzed probe enabled highly specific imaging of SH-SY5Y cells due to the upregulated TG2 expression under hypoxic conditions and improved the intracellular retention efficiency.

Eradicating malignant melanoma remains challenging due to its high rates of metastasis and recurrence following traditional surgery and chemotherapy. Furthermore, long-term chemotherapy could exacerbate drug resistance in tumors.[141,142] In contrast to nucleus-based therapies, targeting the cytoskeleton, which plays a crucial role in cell morphology, adhesion, metastasis, and the fate of resistant cells, offers a promising approach for targeted therapy in malignant or drug-resistant cancers.[143,144] We recently developed an innovative enzyme-induced intracellular self-assembly strategy using the tripeptide

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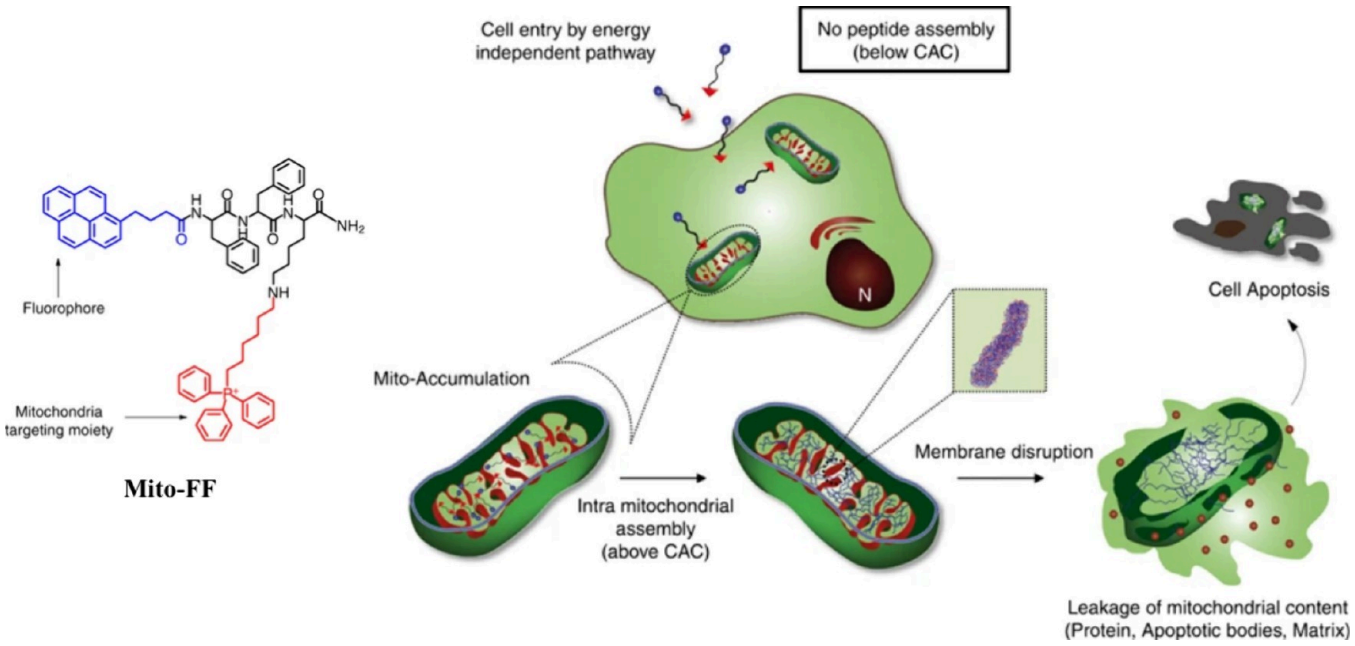

Figure 16. Structural design and intramitochondrial assembly process of Mito-FF. The formation of intramitochondrial fibrils induced mitochondria dysfunction and led to cellular apoptosis. Reproduced with permission from ref 146. Copyright 2017, Nature Publishing Group.

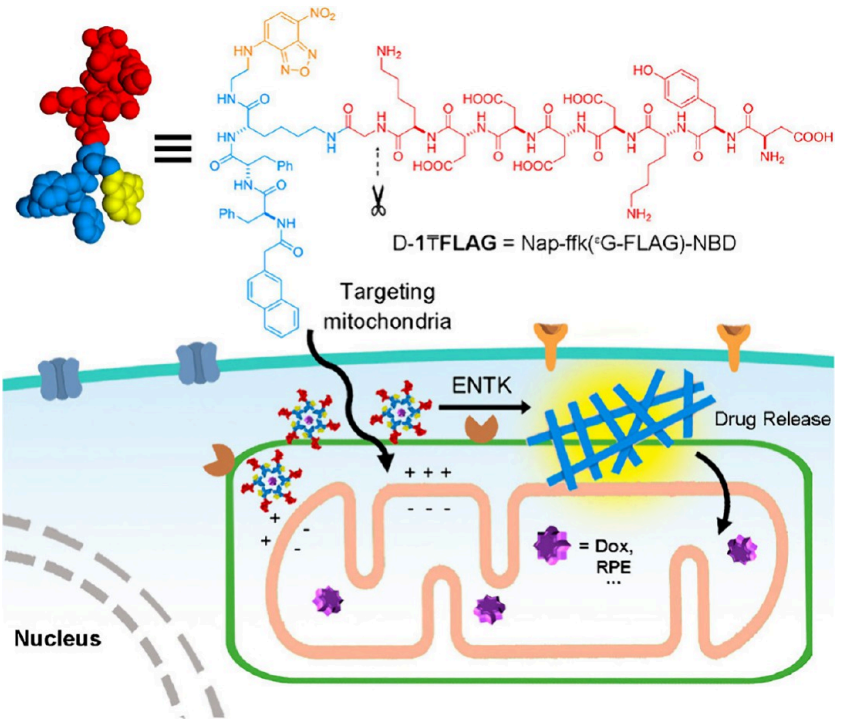

Figure 17. Chemical structure of a representative branched peptide is illustrated, alongside its transformation process from micelles to nanofibers via ENTK cleavage within mitochondria. Reproduced with permission from ref 156. Copyright 2018, American Chemical Society.

phenylalanine−phenylalanine−tyrosine (FFY).[145] As depicted in Figure 15b, FFY was initially oxidized to a melanin-like FFY dimer (mFFY) with a diquinone structure by tyrosinase and subsequently self-assembled into nanoparticles. These nanoparticles interfered with tubulin self-polymerization. The process led to the overproduction of cleaved caspase-3 and cleaved PARP due to mitochondrial dysfunction, effectively reversing drug resistance without the need for chemotherapeutic drugs. *In vivo* results demonstrated that the intracellular oxidation and self-assembly of FFY induce effective intrinsic apoptosis in drug-resistant melanoma by targeting tubulin polymerization and mitochondrial dysfunction. This approach provided new insights into utilizing intracellular self-assembly biomolecules at the subcellular level to overcome the drug resistance.

Spatiotemporal regulation of molecular self-assembly within living cells still presents a significant challenge due to the complexity of intracellular microenvironments. Ryu and co-

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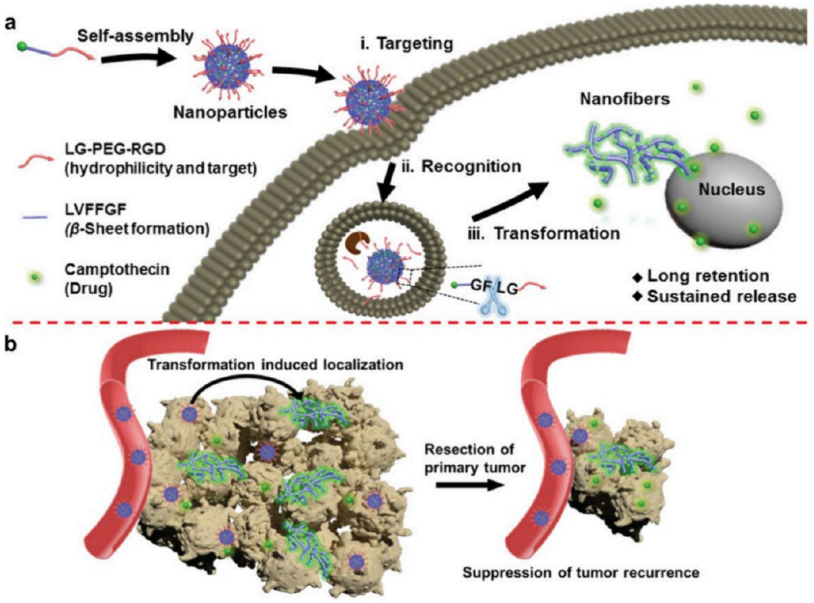

Figure 18. Schematic diagram of transformation-induced localization strategy for inhibition of postsurgical local tumor recurrence. (a) Intracellular self-assembly and transformation process of CPT-LFPR to create a fibrous drug depot. (b) The resulting transformation facilitated prolonged retention of nanodrugs and sustained drug release, thereby suppressing tumor recurrence. Reproduced with permission from 161. Copyright 2019, Wiley-VCH.

workers reported an organelle-localized self-assembly of a peptide-based amphiphilic molecule as an approach to regulate cell fate (Figure 16).[146] The mitochondria-targeting phenylalanine dipeptide (Mito-FF) consisted of three components: (1) Phe-Phe-Lys (FFK) tripeptide as a backbone, (2) fluorescent pyrene butyric acid modified at the N-terminus of FFK to increase the propensity for self-assembly by enhancing hydrophobic and $\pi-\pi$ interactions, and (3) triphenyl phosphonium modified at the amine side chain of lysine to selectively target mitochondria. Within the mitochondria, Mito-FF reached a concentration sufficient to initiate the formation of fibrous nanostructures. This self-assembly mechanism was facilitated by the heightened mitochondrial membrane potential characteristic of cancer cells, which promoted the accumulation of Mito-FF within the mitochondria and subsequent assembly into fibril structures.

2.3. Intracellular Transformation

Self-assembled nanomaterials are extensively utilized in nanomedicine.[147,148] However, the effectiveness of preassembled nanomaterials can be compromised under complicated physiological conditions. This is due to the inherent dynamic nature of self-assembly systems, which can lead to structural changes such as dissociation, aggregation, and transformation.[30,149] Morphology transformation from nanoparticles to nanofibers at the subcellular level can be achieved through various stimuli, enabling versatile bioactivity.[26,30] In addition to morphological changes, nanostructures can undergo transformations in size, surface charge, and shape, aligning with the requirements for biomedical imaging and therapy.

2.3.1. Endogenous Stimulus Induced Transformation. Cellular function can be selectively manipulated through the targeted delivery of bioactive compounds to mitochondria.[150−152] Traditional mitochondria-targeting molecules typically exploit either the mitochondrial membrane potential or the mitochondrial protein-import machinery.[153] These molecules are often lipophilic and cationic, which can result in cytotoxicity due to their accumulation in the mitochondrial matrix.[153] Enzyme-mediated self-assembly offers a promising alternative for mitochondrial targeting.[115,154,155] Xu and co-workers reported an enzymatic cleavage approach using branched peptides with negative charges for targeting mitochondria delivery and nanoparticle transformation.[156] As presented in Figure 17, a FLAG-tag (DDDDK) peptide was designed with a cleavage site for enterokinase (ENTK) and conjugated with a self-assembling peptide. Upon cellular internalization, the hydrophilic FLAG motif underwent intracellular ENTK-mediated cleavage, triggering the transformation of micelles into nanofibers around the mitochondria. This strategy decreased the ratio of off-target mitochondria localization caused by ENTK proteolysis, enabling the delivery of small molecules or proteins into cells within 2 h. In another study, Xu and co-workers reported a similar ENTK-mediated assembly approach targeting mitochondria.[157] Histone protein H2B is normally absent from mitochondria but present in dysregulated cells like cancer cells. MitoFlag, which interacts with the nuclear localization sequence of H2B, prevents H2B from entering the nucleus. ENTK cleavage of the Flag tag from the MitoFlag/H2B complex results in the formation of nanofibers, causing H2B to remain on the mitochondrial surface and facilitating its entry into the mitochondria.

The local tumor recurrence after surgical resection remains a significant in treatment of cancer.[158] Implant materials designed to suppress recurrence *via* sustained-release mechanisms for chemotherapeutic drugs, such as drug eluting wafers, films, and fiber. However, these materials can potentially affect normal tissue the healing, immunity cell infiltration, and drug diffusion.[159,160] Wang and co-workers addressed this issue by designing a peptide-CPT conjugated chemotherapeutic prodrug.[161] The peptide-CPT conjugate, CPT-LVFFGFLG-PEG-RGD (CPT-LFPR), features a targeting motif (PEG-RGD) and a prodrug composed of hydrophobic tumor drug CPT, a β-sheet peptide (LVEF), and a cathepsin B cleavable peptide (GFLG) (Figure 18a). The assembled prodrug nanoparticles

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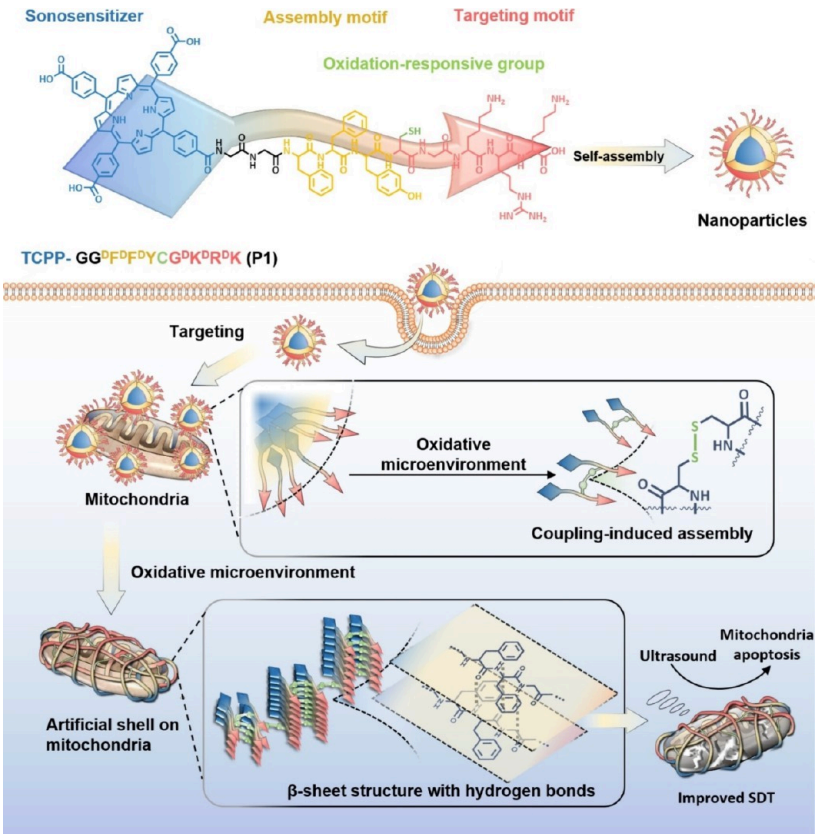

Figure 19. Schematic diagram of a process involving coupling-induced assembly and oxidative induced-transformation. In this process, peptide-porphyrin conjugates (PPCs) self-assembled to nanoparticles that were targeted to mitochondria. The thiol groups in P1 couple to form dimers, which subsequently stack into cross-linked nanofibers under oxidative conditions. Then nanofibers constructed then formed artificial shells on mitochondria. The porphyrin molecules within these shells generated a substantial amount of ROS, leading to apoptosis in the tumor cells. Reproduced with permission from ref 163. Copyright 2024, Wiley-VCH.

selectively accumulated in tumor cells due to the enhanced permeability and retention effect and active targeting of RGD peptide. Upon accumulation, the hydrophilic LG-PEG-RGD motifs were cleaved by a tumor-specific enzyme, cathepsin B, leading to a morphology transformation from nanoparticles to β-sheet fibrous structures. These *in situ* generated fibrous structure served as a long-term drug depot, allowing for the sustained release of free CPT. This approach efficiently inhibited residual microtumor growth and prevented local tumor recurrence following surgical resection (Figure 18b).

The strategy of inducing tumor cell apoptosis by disrupting mitochondrial metabolism is widely used in cancer therapy. Wang and co-workers previously present a mitochondrial-targeted nanosheet (BiOCl) capable of disrupting the mitochondrial membrane potential and inducing cancer cells apoptosis under ultrasound stimulation.[162] However, the therapeutic efficacy of such exogenous interventions was limited due to the transient (~40 ns) lifespan and short diffusion distance of ROS. To address this limitation, Wang and co-workers recently developed an *in vivo* self-assembling peptide system that achieved multivalent cooperative interactions with mitochondrial, thereby enhancing anticancer efficacy. They introduced a coupling-induced assembly strategy that constructed an artificial shell on the mitochondria of tumor cells.[163] As shown in Figure 19, the peptide porphyrin conjugate (PPC) was consisted several modules: mitochondria-targeting and endogenous oxidation-responsive module (CGDKDRDK), a self-assembling module (FFY), and the sonosensitizer porphyrin (TCPP). In the oxidative environment surrounding the mitochondria, the thiol group on PPC was oxidized, forming covalent disulfide bonds that induce the formation of dimers. The increased molecular rigidity prompted the dimers of FFY, successfully constructing covalently cross-linked nanofibers, referred to as artificial shells, on the mitochondria surface. This CIA strategy significantly enhanced the disturbing of mitochondrial membrane potential and induced tumor cell apoptosis through ROS generation under sonication, thereby improving the therapeutic outcome.

pH changes during cellular entry can serve as an endogenous trigger for the self-assembly or morphological transformation of a pH-responsive nanomaterial.[36] Yu and co-workers reported a tumor microenvironment-adaptable self-assembly of pentapeptides, regulated by the pH-sensitive cis/trans isomerization of 4-amino-proline (Amp) amide bonds, to enhance drug delivery and PDT efficacy.[164] This design was inspired by studies showing that human protein β2-microglobulin contains a proline residue with a stable cis conformation in its native structures but undergoes misfolding and aggregation into amyloid fibrils due to cis-to-trans amide isomerization of proline. This finding led to the idea of incorporation proline analogs with adaptable trans/cis-amide-isomer isomerization ratios into short peptides to manipulate their self-assembly. The peptide AmpF, containing a central Amp residue flanked by two diphenylalanine segments, was engineered to regulate its self-assembly based on pH-sensitive

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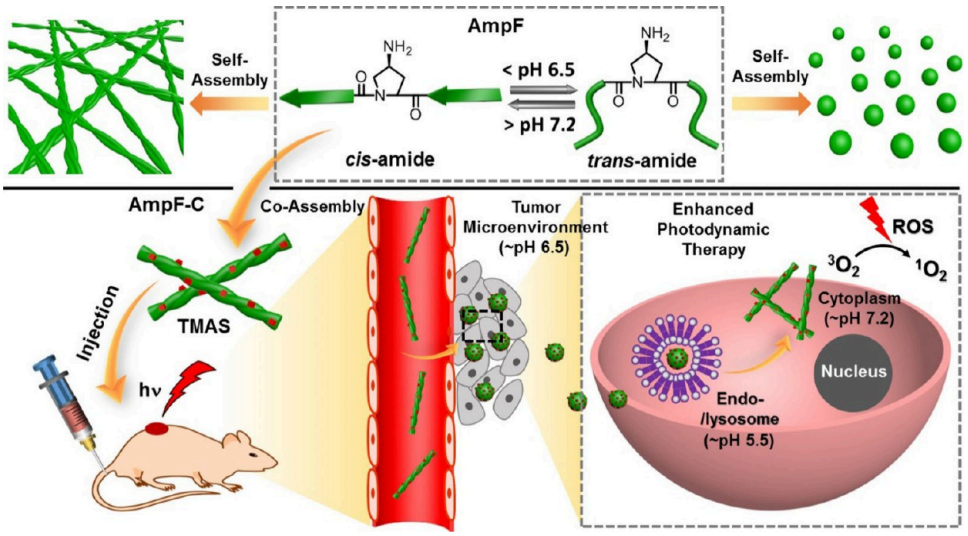

Figure 20. Schematic diagram of peptide-based nanomedicine design. The cis/trans-isomerization of Amp amide bonds enabled the reversible morphological transition of AmpF between superhelices and nanoparticles. AmpF and AmpF-C coassembled to form TMAS nanomedicines, which underwent a reversible morphological change in response to the intracellular pH gradients. Reproduced with permission from ref 164. Copyright 2019, American Chemical Society.

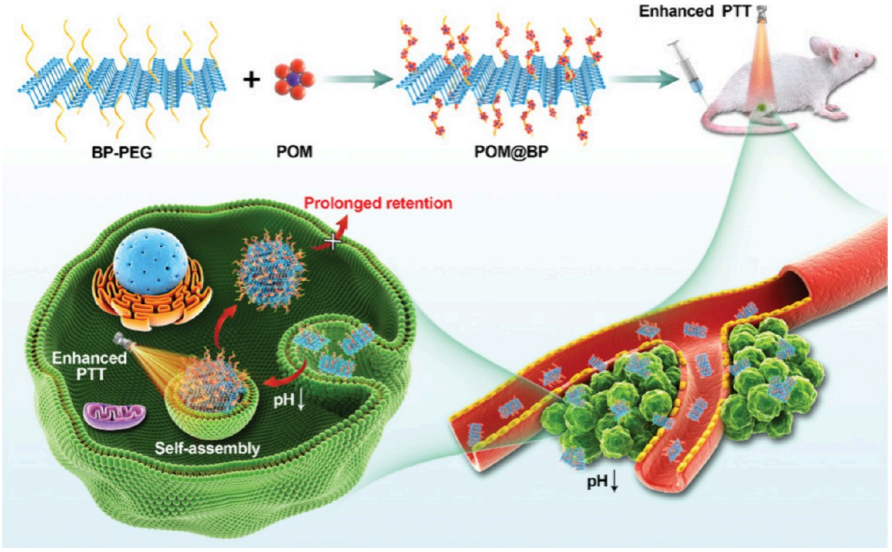

Figure 21. Schematic diagram of acid-responsive intracellular self-assembly and transformation of POM@BP, which enhanced tumor retention and photothermal therapy efficacy. Reproduced with permission from ref 165. Copyright 2020, Wiley-VCH.

cis/trans-amide isomerization. As shown in Figure 20, the nanomedicine was synthesized by assembling the pentapeptide FF-Amp-FF (AmpF) and a conventional photosensitizer, chlorin e6 (Ce6), attached to the N-terminus to AmpF, forming AmpF-C. This tumor microenvironment-adaptable self-assembly (TMAS) system underwent a series of pH-dependent morphological transitions as it traveled through different cellular compartments. In the bloodstream (pH 7.4), the system formed superhelices, which transformed into nanoparticles around tumor tissues (pH 6.5), internalized into endo- and lysosomes (~pH 5.5), and then reassembled into nanofibers within cancer cells (pH 7.2−7.6). Compared to morphology-persistent nanomedicines, the TMAS nanomedicines exhibited prolonged circulation *in vivo* and improved tumor accumulation, resulting in a lower half lethal dose for cancer cells.

As presented in Figure 21, Zhang and co-workers performed an acid-activated smart self-assembly combining black phosphorus (BP) with polyoxometalate (POM), resulting in the formation of POM@BP.[165] In an acidic tumor microenvironment, POM particles decorated the surface of BP nanosheets, facilitating the self-assembly of POM@BP into larger nanoparticles. This acid-triggered assembly significantly improved the light absorption capacity of the BP PTT. The POM@BP system demonstrated prolonged tumor retention, thereby enhancing PTT efficiency by the smart self-assembly of BP.

Wang and co-workers presented an organelle-targeting morphology transformation platform based on ROS-responsive polymer−peptide conjugate, designed to undergo morphological changes for enhanced cooperative interaction with mitochondria at tumor sites.[166] As depicted in Figure 22, P1 was composed of three parts: (1) a mitochondria-targeting

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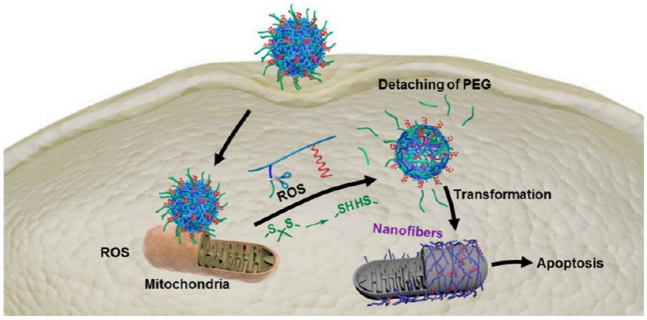

Figure 22. Synthetic route of ROS-responsive polymer−peptide conjugation and its morphological transformation in mitochondria. Reproduced with permission from ref 166. Copyright 2019, American Chemical Society.

cytotoxic peptide (KLAK); (2) a β-sheet-forming peptide (KLVFF) linked with hydrophilic poly(ethylene glycol) (mPEG) via ROS-cleavable thioketal bond; and (3) a poly(vinyl alcohol) backbone. Upon cleavage of the thioketal linker by elevated ROS levels near the mitochondria, the amphiphilic polymer−peptide conjugate self-assembled into nanoparticles. The mitochondria-targeting KLAK peptide, along with the morphology transformation, enhanced the accumulation of the nanoparticles in tumor tissues, leading to increased inhibition of tumor growth.

Besides self-assembly of synthesized molecules within cells, naturally occurring biomolecules also widely undergo an intracellular self-assembly process to construct cell components and regulate cellular functions. Fibronectin, a key extracellular matrix protein, self-assembles into a fiber network that regulates cell adhesion, migration, and phagocytosis.[167,168] The initiation of fibronectin self-assembly commences with binding between fibronectin and its receptor, integrin, on the cell surface. This interaction event disrupted the equilibrium between hydrophobic and hydrophilic interactions within fibronectin, leading to a conformational change in the fibronectin. Subsequently, fibronectin exposed the self-assembling sequence and self-assembled into fibrous structures. This process is known as binding induced fibrogenesis (BIF).[169] As illustrated in Figure 23, Wang and co-workers reported a BIF peptide (Bis-pyrene-KLVFF-VNTANST) that forms an *in situ* fiber network to prevent improper self-assembly of vimentin in breast cancer cells.[170] The KLVFF sequence, acting as an assembly motif, facilitated the formation of nanofibers in BFV via hydrogen bonding. The VNTANST sequence, known for its targeting ability, specifically bonded to vimentin and initiated the fibrillogenesis process. The incorporation of bis-pyrene into BFV contributed to the formation of BFV nanoparticles, utilizing its hydrophobic nature and $\pi-\pi$ stacking interactions. When BFV nanoparticles are internalized into tumor cells, the BIF peptide banded to vimentin and became a BFV fiber network. The synthetic peptide fiber network disrupted vimentin skeletonization, inhibiting tumor cell migration and invasion. *In vivo* experiments demonstrated that the BIF peptide fiber network effectively reduced tumor growth and metastasis while modulating the tumor microenvironment.

2.3.2. Exogenous Stimulus Induced Transformation.

Light is an ideal tool for manipulating cellular behaviors because of its wide availability, noninvasive nature, low cost, and environmental friendliness. It can also be precisely tuned for spatial and temporal control.[41] Zou and co-workers reported a light-triggered molecule that enabled spatiotemporal control of self-assembly morphology within living cells.[171] As shown in Figure 24, the platform comprised a light-controlled unit, 3-methylene-2-(quinolin-8-yl) isoindolin-1-one, which could alter the hydrophilic/lipophilic balance through the changing molecular conformation and a peptide capable of rapid reassembly through internal hydrogen bonding interactions. Nanoparticles containing this light-responsive units underwent morphological transformation on the cell surface and within lysosomes upon irradiation at a wavelength of 365 nm. The transformation disrupted the lysosomal membrane, enhancing the escape of lysosome and

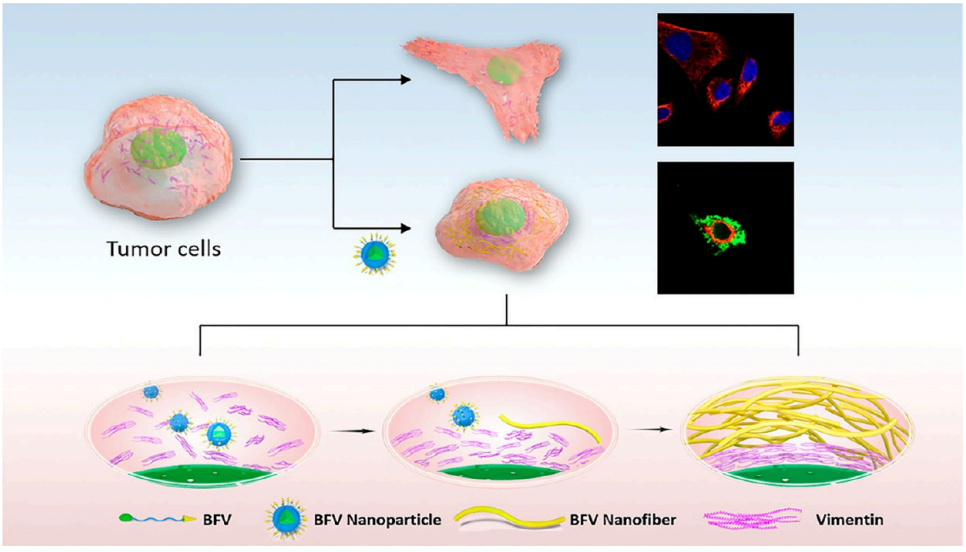

Figure 23. Schematic diagram of the transformation process based on binding-induced fibrillogenesis of the peptide bis-pyrene-KLVFF-VNTANST (BFV). BFV nanoparticles banded to the vimentin, forming fibrous structures from the continuous internalization of BFV nanoparticles. This results in the encapsulation of vimentin and the inhibition of vimentin skeletonization. Reproduced with permission from ref 170. Copyright 2021, American Chemical Society.

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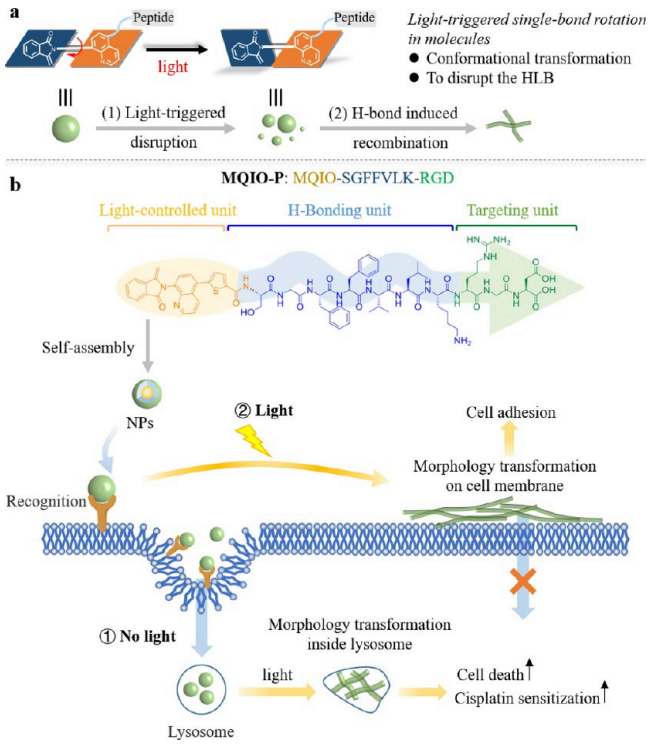

Figure 24. Illustration diagram of light-triggered self-assembly and transformation by molecular conformation changes and H-bond interactions. (a) Illustration diagram for single-bond rotation in molecules and morphology transformation from nanoparticles to nanofibers induced by light. (b) Molecular structure of MQIO-P and its self-assembly and transformation process formed by light on the cell surface and inside the lysosome. Reproduced with permission from ref 171. Copyright 2022, American Chemical Society.

inducing cell apoptosis, thereby increasing cisplatin sensitization.

As illustrated in Figure 25, Zhu and co-workers developed a tumor-targeting and caspase-3-responsive nanoparticle system composed of a diacetylene-containing lipidated peptide amphiphile (DEVD-DLPA) and a mitochondria-targeting photosensitizer.[172] Upon cellular internalization and exposure to laser irradiation, mtROS levels increased, triggering apoptosis by overexpression of caspase-3. Caspase-3 then cleaved the DEVD-DLPA nanoparticles into diacetylene-GDEVD (blue sphere with a short blue tail) and RRRRGDS moieties, leading to the disassembly of the NP and the subsequent release of the photosensitizer. In the presence of mtROS, diacetylene-GDEVD self-assembled into nanofibers, either moniliform or dense smooth fibers, on mitochondrial membranes. These locational nanofibers caused significant mitochondrial damage, amplifying mtROS production and promoting a self-circulatory apoptotic cycle, which ultimately enhanced the antitumor therapeutic efficacy.

3. CHALLENGES AND FUTURE PERSPECTIVES

Subcellular compartments consist of organelles and the surrounding microenvironment, offering unique reaction conditions for various biochemical processes. Recent advances in precise regulation within cells through intracellular polymerization, self-assembly, and transformation have been explored. The approaches described above enable interdisciplinary collaborations between chemistry and biology for enhanced therapeutic outcomes. In this review, we have summarized the latest developments in polymerization, assembly, and transformation within living cells to achieve precise regulation of related bioactivities. Despite the considerable progress in this field, many challenges remain unsolved, such as limited monomer candidates, complex microenvironments, and unavoidable side reactions. Herein we anticipate a series of challenges and opportunities that lie ahead in the area of intracellular polymerization, self-assembly, and transformation materials for precise manipulation of bioactivities at the subcellular level:

(1) There are certain risks associated with the use of nanomaterials, especially their potential biological toxicity. The toxicity of nanomaterials within cells mainly arises from their interactions with proteins, membranes, and other biomolecules, which could lead to protein dysfunction, cell membrane damage, and DNA damage. Besides possible interactions with biomolecules, degradability is also a crucial factor when studying the toxicity of nanomaterials. If nanomaterials cannot be degraded and eliminated from the body, they may accumulate in specific tissues and organs for a long time, leading to inflammatory responses, oxidative stress, or even organ dysfunction. Meanwhile, even with satisfactory biodegradability, it is necessary to consider the potential toxicity of their degradation products. Some studies have shown that certain degradation products may exhibit cytotoxicity and affect the normal physiological functions of cells. Therefore, comprehensive toxicological evaluations of the degradation process and degradation products should be conducted during the biomedical applications of intracellular polymerization, assembly, and transformation.

(2) The core of precision medicine lies in developing personalized treatment plans based on an individual's genomic information, biomarkers, and disease characteristics, which not only enhances treatment efficacy but also reduces unnecessary side effects. In this context, discovery and identification of biomarkers and drug targets have become focal points for developing biomedical nanomaterials. Biomarkers are biological molecules within an organism that can reflect disease states or treatment progress, such as genes, proteins, and metabolites. On the other hand, the targets of drugs are specific molecules or pathways in the body that aim to modulate metabolism in the disease. Identifying the right drug targets is crucial for developing effective therapies. RNA sequencing is a high-throughput technology that comprehensively reveals changes in gene expression, aiding researchers in discovering new biomarkers and drug targets. Upon establishing biomarkers or targets within cells, intracellular synthesized biomaterials could be designed with specific structures to bind with above biomarkers or targets with enhanced therapeutic outcomes.

(3) A major drawback of traditional therapy is the lack of selectivity, which could cause harm to normal cells or tissues. Despite some success in improving the targeting of drugs and reducing side effects, there are still limitations of intracellular reaction-based strategies. As current designs are mainly dependent on a reaction triggered by a single parameter, the varying microenvironmental characteristics of different cell types at

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JACS Au 2024, 4, 4162−4186

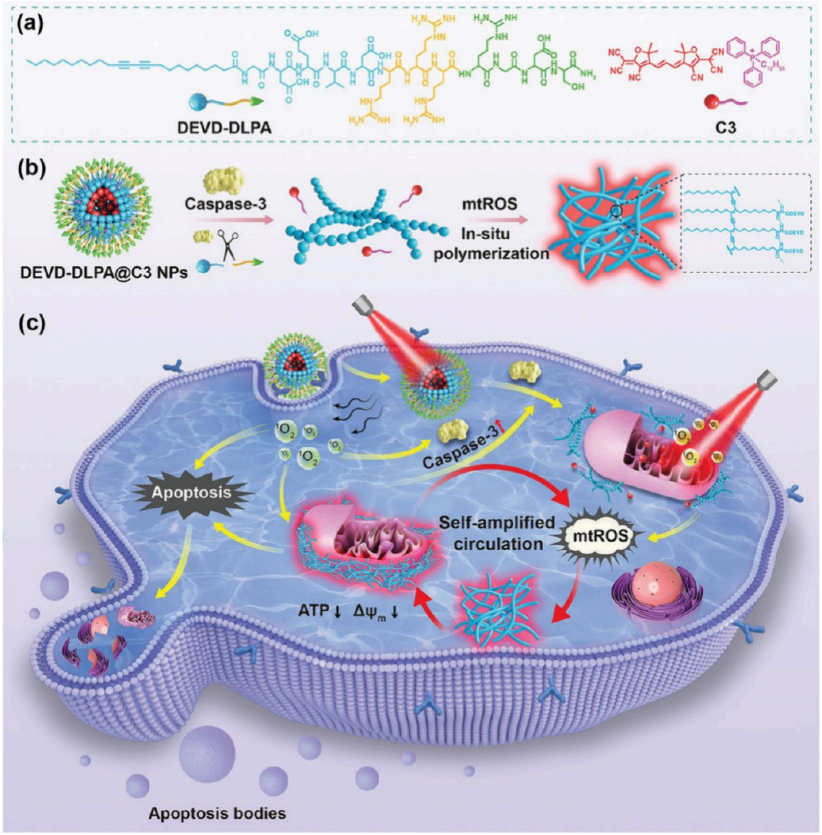

Figure 25. Schematic illustration of (a) molecular structures of DEVD-DLPA and (b) morphological transformation induced by laser irradiation and generated ROS. (c) Schematic illustration of PDT induced intracellular polymerization and self-circulatory amplification of tumor therapy caused by mtROS. Reproduced with permission from ref 172. Copyright 2022, Wiley-VCH.

various time points allow the precise control of intracellular synthesis. To address these issues, it is of great value to develop intracellular synthesis triggered by combined parameters to meet the complicated physiological environments. For example, light, electricity, ultrasound, pH and enzymes could be utilized as cascade triggers to initiate intracellular polymerization, assembly, and transformation with designated biofunctions.

(4) Traditional web-lab synthesis usually requires a series of trial-and-error experiments to determine the optimal material composition and structure, which is time-consuming, labor-intensive and difficult to achieve breakthrough progress in a short period of time. Currently, with the fast developments of artificial intelligence (AI), it is possible to speed up the intracellular polymerization, assembly, and transformation with enhanced outcomes through analyzing a large amount of experimental data and identify potential patterns and properties in a short time with machine learning algorithms. For example, AI can be used to screen and evaluate the impact of different material combinations within cells, thus accelerating the development process of intracellular synthesized materials. By analyzing the complex relationships between material properties and cell responses, researchers can design experimental methods more accurately and improve the success rate and efficiency of experimental outcomes. In addition, AI can be used to predict the metabolism behavior and biocompatibility of drugs in human body, which is of great significance for personalized medical treatment.

(5) Currently, the biomedical applications of intracellular synthesis were focused on cancer treatment. Meanwhile, it is worth noting that most disease are caused by the imbalance within the body's homeostasis, which could also be regulated through intracellular polymerization, assembly, and transformation. In the state of imbalance, various inducers in the microenvironment can lead to abnormal metabolic products or signaling pathways within cells, which can become targets for intracellular reactions. Therefore, applying these intracellular regulatory strategies to other types of diseases, such as cardiovascular diseases, microbial infections, and neurodegenerative diseases, holds significant potential and promise. For instance, in cardiovascular diseases, the dysfunction of vascular endothelial cells and inflammatory responses are crucial factors leading to atherosclerosis. By designing specific intracellular reaction mechanisms, it is possible to suppress the expression of inflammatory factors and restore the normal function of vascular endothelial cells, thereby slowing down or even halting the progression of atherosclerosis. In recent years, significant advancements have been made in fields such as synthetic biology, pharmacology, and toxicology. These advancements provide us with powerful tools and methods, making it increasingly feasible and promising to apply intracellular regulatory strategies in clinical settings.

https://doi.org/10.1021/jacsau.4c00849
JACS Au 2024, 4, 4162−4186

In summary, intracellular polymerization, self-assembly, and transformation have offered great potential for regulating subcellular functions. However, a series of challenges remain, including possible biotoxicity, unregulated biodegradability, and relatively unsatisfactory therapeutic outcomes. To overcome these obstacles, it is crucial to apply new theories and techniques to design and synthesize biomaterials intracellularly. For instance, multiple experimental parameters could be utilized as cascade triggers to initiate intracellular polymerization, assembly, and transformation with the designated biofunctions. In addition, the integration of artificial intelligence with intracellular synthesis offers powerful new methods to accelerate the development of innovative biomaterials within cells. Looking to the future, these approaches are poised to revolutionize various fields by enhancing treatment efficacy, minimizing side effects, and ultimately improving the overall quality of life of patients. We hope that this Perspective serves as a prologue to scientists in both academia and industry to advance the research of regulating bioactivities at the subcellular level through intracellular polymerization, assembly, and transformation.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by National Natural Science Foundation of China (52222306, 22075212, 21925505, 22335005, and 22305177), Natural Science Foundation of Shanghai (20ZR1443200) and international scientific collaboration fund (23520710900) and Shanghai Rising-Star Program (Sailing, 23YF1433000) of Science and Technology Commission of Shanghai Municipality, the fellowship of China Postdoctoral Science Foundation (2022M720107, GZB20230517, 2024T170668), Shanghai "Super Postdoc" Incentive Plan of Shanghai Municipal Bureau of Human Resources and Social Security (2022568). J.D. is the recipient of National Science Fund for Distinguished Young Scholars.

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