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Advancements and perspectives on organelle-targeted fluorescent probes for super-resolution SIM imaging

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As a cutting-edge super-resolution imaging technique, structured illumination microscopy (SIM) has been widely used in cell biology research, especially in the analysis of subcellular organelles and monitoring of their dynamic processes. Through multiple illumination and reconstruction processes, SIM breaks through the resolution limitations of traditional microscopes and can observe the fine structures within cells in real time with nanoscale resolution. This provides strong technical support for in-depth analyses of molecular mechanisms, organelle functions, signaling networks, and metabolic regulatory pathways within cells. In recent years, super-resolution imaging technology, as a novel imaging technique, has made significant research progress in subcellular fine structure imaging by combining fluorescent probes. However, there is an urgent need for a more comprehensive review of the use of fluorescent probes in various organelles (such as mitochondria, lysosomes, lipid droplets, cell membranes, and the endoplasmic reticulum) by SIM imaging technology. Therefore, this review provides a comprehensive overview of the latest research progress in observing different organelles using small-molecule fluorescent probes by SIM imaging. At the same time, we also discussed the challenges and prospects of current SIM technology in dynamic process observation, including further improvement of resolution, photobleaching issues in long-term observations, and innovation in multimodal imaging. Overall, this review fills the research gap in this important field by providing a comprehensive, multi-organelle-targeted fluorescent probe perspective, combined with SIM imaging technology, particularly providing readers with new ideas and insights at multiple levels, such as technology, design, application, and disease research.

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1. Introduction

Organelles are membrane structures or structural units with specific functions within cells, typically existing in the cytoplasm in different forms and functions, playing a key role in maintaining the normal physiological activities of cells.[1] They are the main sites for intracellular biochemical reactions and play essential roles in various life activities such as cell division, energy metabolism, protein synthesis, and material transport. Typical organelles include the mitochondria, endoplasmic reticulum, Golgi apparatus, lysosomes, lipid droplets, nucleus, *etc.* Each organelle has a unique structure and function, and the coordinated operation of these organelles ensures the normal function and life activities of cells.[2–5] With the deepening of biological research, the study of organelles is not limited to their basic biological functions, but has also expanded to the role of organelles in disease development, especially in pathological processes such as cancer and neurodegenerative diseases, where the disruption of the functional homeostasis of the organelles correlates significantly with the emergence of pathological states.[6–8] Therefore, a deep understanding of the structure, function, and role of organelles in physiology and pathology is of great significance for revealing the mysteries of life and exploring new strategies for disease treatment.

In recent years, with the rapid development of imaging technology, especially the emergence of super-resolution microscopy, scientists have been able to observe the dynamic changes of organelles and the microenvironment inside cells at higher resolutions, thus opening up a new perspective for organelle research. This provides strong technical support for in-depth exploration of the functions of organelles and their applications in biomedical fields. However, with the advancement of super-resolution technology, various super-resolution microscopy techniques have emerged, such as Structured Illumination Microscopy (SIM), stimulated emission depletion (STED) Microscopy, Single Molecule Localization Microscopy (SMLM), and random optical reconstruction microscopy (STORM).[9–12] These technologies have broken through the diffraction limit of traditional microscopes, enabling super-

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Table 1 Comparison of different super-resolution imaging techniques

Tools	Applicability	Advantage	Disadvantage	References
SIM	Real-time dynamic imaging and live cell research	Fast imaging, simple equipment, low light damage, and suitable for dynamic imaging	Relatively low resolution and high requirements for fluorescence signals	*Nat. Methods*, 2018, **15**, 1011–1019;[9] *Trends Anal. Chem.*, 2023, **169**, 117370 (ref. 29)
STED	High-resolution static imaging and molecular structure observation	High resolution, direct imaging,and no need to reconstruct images	Slow imaging speed, light damage, and high sample requirements	*Adv. Drug Delivery Rev.*, 2024, **210**, 115330 (ref. 15)
SMLM	Single-molecule behavior observation and ultra-high resolution molecular distribution research	Extremely high resolution and precise molecular localization	Slow imaging speed, fluorescence bleaching issues, and complex sample preparation	*Chem. Soc. Rev.*, 2023, **52**, 942–972 (ref. 49)
STORM	Ultra high resolution imaging and observation of protein complexes and nanoscale structures	Extremely high resolution, no need for complex equipment, and molecular-level positioning	Slow imaging speed, photo bleaching issues, and high data processing requirements	*Nat. Methods*, 2006, **3**, 793–796 (ref. 12)

resolution imaging at the subcellular level and providing more detailed visualization of cell structures. Among many super-resolution imaging techniques, SIM stands out with its unique advantages. Compared with other methods, SIM has a wider choice of fluorescent dyes, higher temporal resolution, and significantly higher fluorescent photon utilization, which has led to its widespread application in subcellular imaging (Table 1).[13–16] Structured Illumination Microscopy (SIM) is a super-resolution microscopy imaging technique that breaks through the diffraction limit of traditional optical microscopes and achieves higher-resolution imaging by illuminating the sample with strip light and reconstructing the image using mathematical algorithms. Compared with traditional fluorescence microscopy, SIM technology can provide clearer and more detailed image information at the cellular and subcellular levels, providing a powerful tool for bioscience research. Specifically in the observation of complex cellular structures and their dynamic changes, SIM technology shows excellent performance and a wide range of application prospects.

The selection of fluorescent probes is crucial for obtaining high-quality images in super-resolution imaging. In recent years, researchers have developed various types of fluorescent probes to meet different imaging needs. Therefore, to develop fluorescent probes suitable for SIM imaging, the following design requirements should be met: (1) small molecule probes should have a high quantum yield in super-resolution imaging to ensure signal strength;[17,18] (2) probes need to have stable optical properties to support long-term dynamic imaging;[19] (3) the probe should have narrow spectral characteristics and consider a larger Stokes shift to improve the signal-to-noise ratio;[20] (4) it should contain functional groups that can specifically bind to subcellular molecules;[21–23] (5) the probe should also include organelle targeting tags to ensure enrichment in specific organelles.[24–26] The development and use of fluorescent probes for SIM-based imaging has become a hot research topic. However, to our knowledge, most of the relevant reviews currently reported are about super-resolution imaging techniques in a broad sense or only discuss a small portion of the applications of fluorescent probes targeting specific organelles (such as mitochondria, lysosomes, *etc.*) by SIM imaging technology. Therefore, a comprehensive review of SIM imaging fluorescent probes for organelles such as mitochondria, the endoplasmic reticulum, lysosomes, lipid droplets, and cell membranes is still urgently needed. This review systematically reviews the cutting-edge advances in resolving the dynamic processes of organelles using SIM imaging since 2021, focusing on the structural features, functional regulation, and dynamic behavioural patterns of different organelles during the cell life cycle. Meanwhile, this paper discusses the existing technical bottlenecks in this field and explores the future development trends and potential challenges. We hope this review will offer a broader perspective for the development of novel super-resolution fluorescent probes for research on subcellular structure-related diseases, thereby providing new insights and approaches for disease diagnosis and treatment.

2. The principle of SIM imaging technology

Structured Illumination Microscopy (SIM) is a super-resolution imaging technique that breaks through the diffraction limit of traditional optical microscopes by illuminating samples with structured light and combining mathematical reconstruction methods to enhance image resolution. The working principle of SIM is based on breaking through the optical diffraction limit.[27,28] The resolution of traditional optical microscopes is limited by diffraction effects, typically around 200 nm. SIM imaging technology utilizes spatially structured light beams to excite fluorescence, creating a mixed frequency of excitation patterns and fluorophore densities, and carries high-frequency information that is usually invisible to the visible low-pass frequency range of the microscope. By altering the direction and phase of the patterns, fluorescence results are recorded, and multiple image datasets are processed to extract the high-frequency information, enabling the reconstruction of super-resolution images[29] (as shown in Fig. 1). Compared with other super-resolution imaging techniques such as STED and

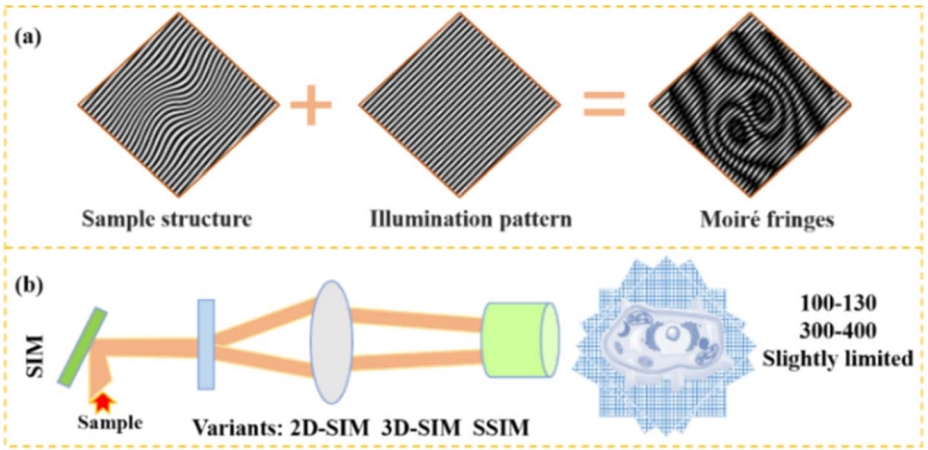

Fig. 1 Basic principles of SIM technology. (a) Schematic diagram of the process of generating Moiré fringes. (b) Simulation diagram of the SIM structured illumination microscope setup. Reproduced with permission from Elsevier, copyright 2023, as cited in ref. 29.

STORM, SIM has a faster imaging acquisition speed and lower phototoxicity, making it particularly suitable for biological samples that require dynamic observation, such as molecular activity in living cells. SIM technology can provide both high resolution and good imaging efficiency, making it highly promising for applications in fields such as cell biology and molecular biology.[30,31] Although SIM has certain limitations in improving resolution, it still provides an important tool for researchers to promote research progress in fields such as life sciences and materials science.

3. Design strategies of fluorescent probes for SIM imaging of different organelles

Most super-resolution probe designs include a large conjugated structure as a fluorescent group, which can emit efficient fluorescence signals under excitation light irradiation, thereby achieving high-resolution imaging. This fluorescent group is usually chemically linked to a specific binding group, which can

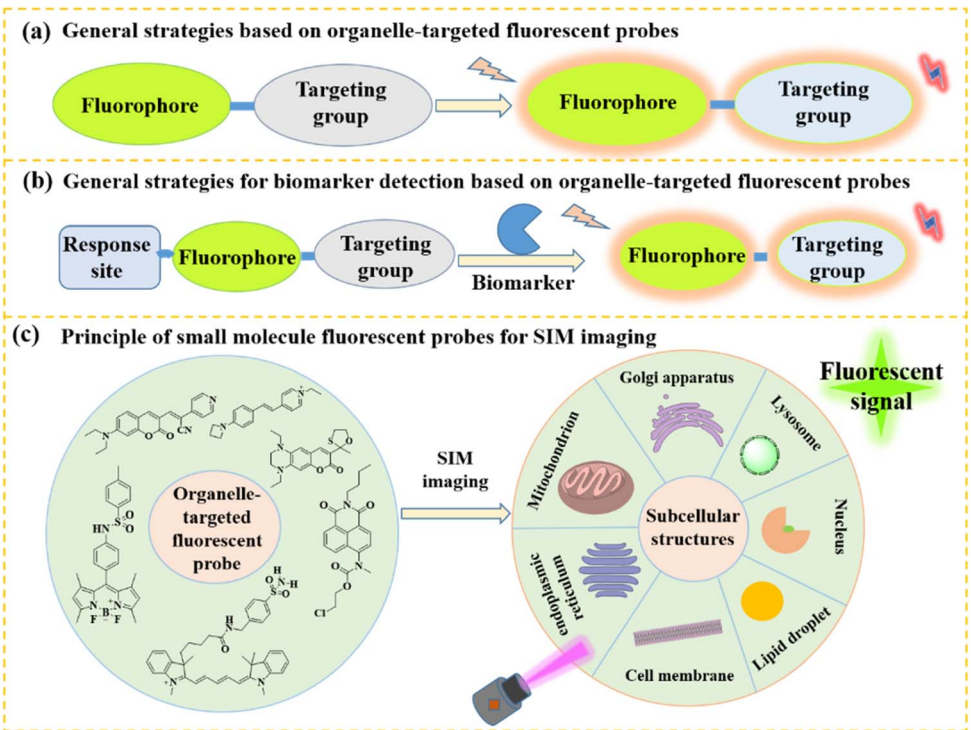

Fig. 2 Strategies for designing fluorescent probes targeting different organelles for SIM imaging. (a) General strategies based on organelle-targeted fluorescent probes. (b) General strategies for biomarker detection based on organelle-targeted fluorescent probes. (c) Principle of small-molecule fluorescent probes in SIM imaging.

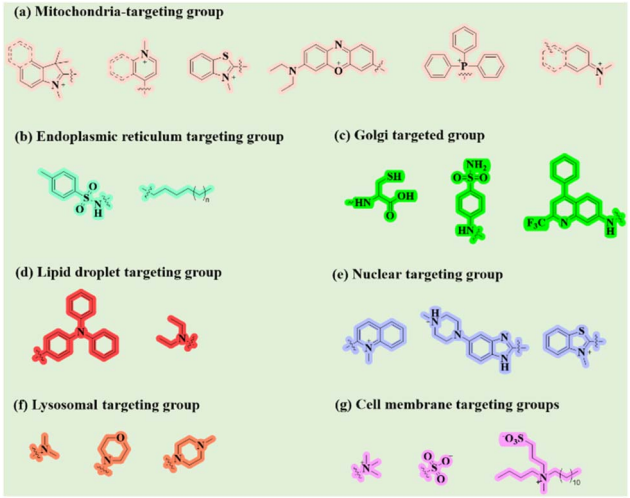

Fig. 3 Common targeting organelle groups used for designing probes targeting different organelles: (a) mitochondria; (b) the endoplasmic reticulum; (c) the Golgi apparatus; (d) lipid droplets; (e) nucleus; (f) lysosomes; (g) cell membrane.

bind to specific molecules or organelle targeting groups within the cell.[32–34] Through this design, probes can accurately identify and accumulate in target organelles or subcellular structures. For example, probes can target organelles such as mitochondria, lysosomes, the endoplasmic reticulum, and lipid droplets, and accumulate in specific organelles after binding, providing clear super-resolution imaging to help researchers observe complex molecular dynamics and organelle functions within cells[35–38] (as shown in Fig. 2).

3.1 Targeted units of different organelles

In the development of organelle-specific fluorescent probes for super-resolution microscopy (SIM), selecting appropriate targeting moieties is essential. These moieties ensure precise localization of the probes to particular organelles, including mitochondria, lysosomes, the endoplasmic reticulum, the Golgi apparatus, nucleus, lipid droplets, and plasma membrane, facilitating high-resolution, high-contrast imaging in SIM (as shown in Fig. 3). For example, probes targeting mitochondria (such as TPP, rhodamine derivatives, *etc.*) can combine their negative membrane potential characteristics to achieve highly selective labeling, thereby revealing the dynamic changes of mitochondria in energy metabolism, oxidative stress, and cell apoptosis; probes for lysosomes, such as morpholine and weak alkaline groups, rely on their acidic environment (pH 4.5–5.0) to achieve targeting, which is helpful for studying autophagy, lysosomal storage disorders, and drug degradation processes; and nuclear probes (such as Hoechst, DAPI, *etc.*) achieve localization by binding to DNA grooves, which can be used to clearly observe gene expression, chromatin dynamics, and cell cycle changes; endoplasmic reticulum probes (such as sulfonylureas and Sec61 targeting peptides) can monitor key biological processes such as protein folding, calcium signaling, and endoplasmic reticulum stress. In addition, specific probes for the Golgi apparatus (such as targeting groups for galactosyltransferase), lipid droplets (such as neutral lipid affinity probes), and cell membranes (such as long-chain lipid modifications) also provide important tools for studying membrane transport, lipid metabolism, and intercellular communication. Through this targeted design for specific organelles, fluorescent probes can not only improve the localization accuracy and resolution of imaging, but also reduce non-specific background signals, thereby helping researchers to more accurately analyze the spatiotemporal dynamic relationships of different structures and functions within cells. These advances not only drive the development of fields such as cell biology, molecular pathology, and drug screening, but also provide powerful imaging tools for disease mechanism research (such as neurodegenerative diseases, cancer metabolic reprogramming, *etc.*) and precision medicine.

3.1.1 Fluorescent probes for mitochondrial SIM imaging. Mitochondria are intracellular organelles known as the "power plants of the cell". They are an important part of most eukaryotic cells and are responsible for the production and storage of

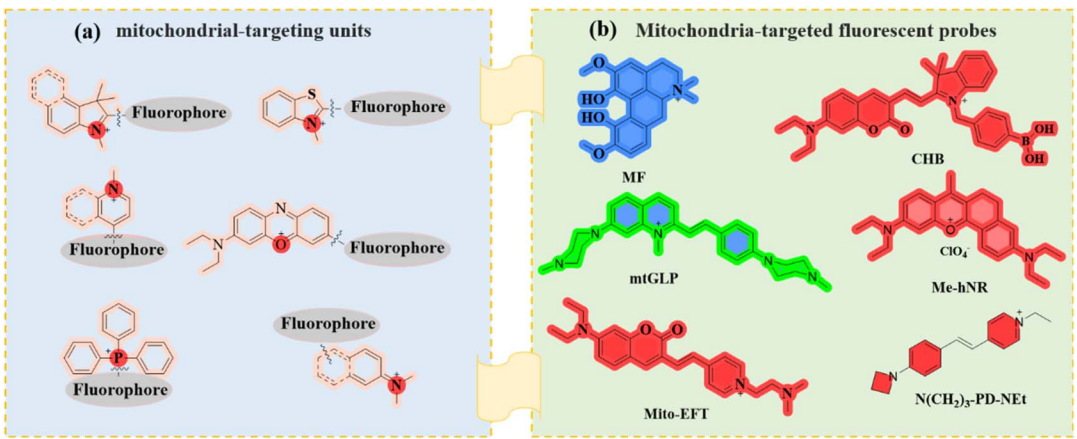

Fig. 4 Design of mitochondria-targeted fluorescent probes. (a) Targeting motifs that commonly make up mitochondria-targeted fluorescent probes. (b) Representative mitochondrial fluorescent probes.

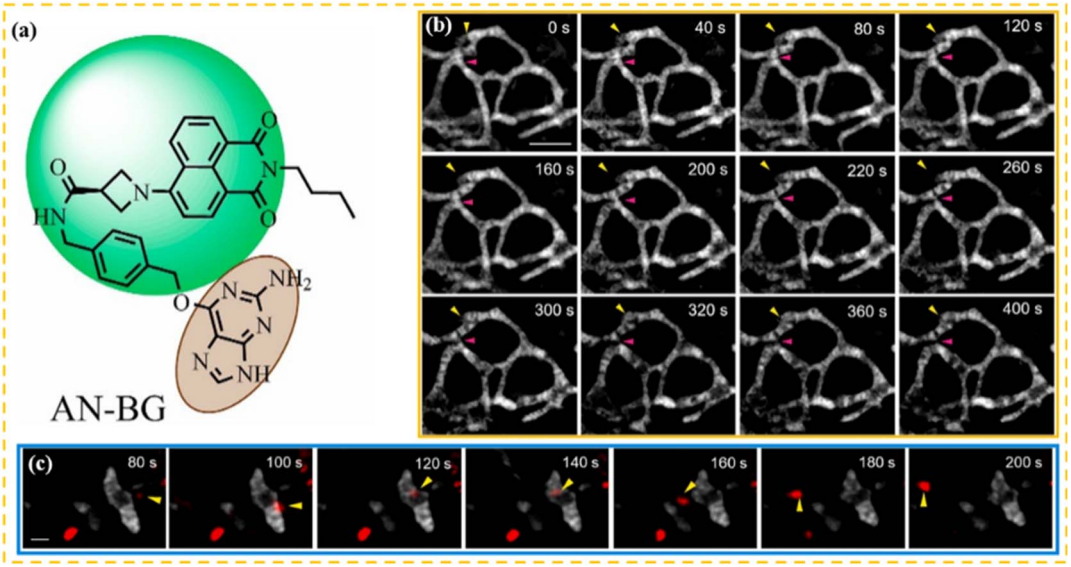

Fig. 5 Dynamic SIM imaging of probe **AN-BG** in mitochondria as well as mitochondria and lysosomes in live cells. (a) Chemical structure of probe **AN-BG**. (b) SIM imaging of dynamic processes in mitochondria of live HeLa cells with probe **AN-BG**. (c) SIM imaging of dynamic processes in HeLa cells with Lysol-Tracker Red (red) and SNAP-labelled fluorescent probe **AN-BG** (grey). Reproduced with permission from Elsevier, copyright 2021, as cited in ref. 55.

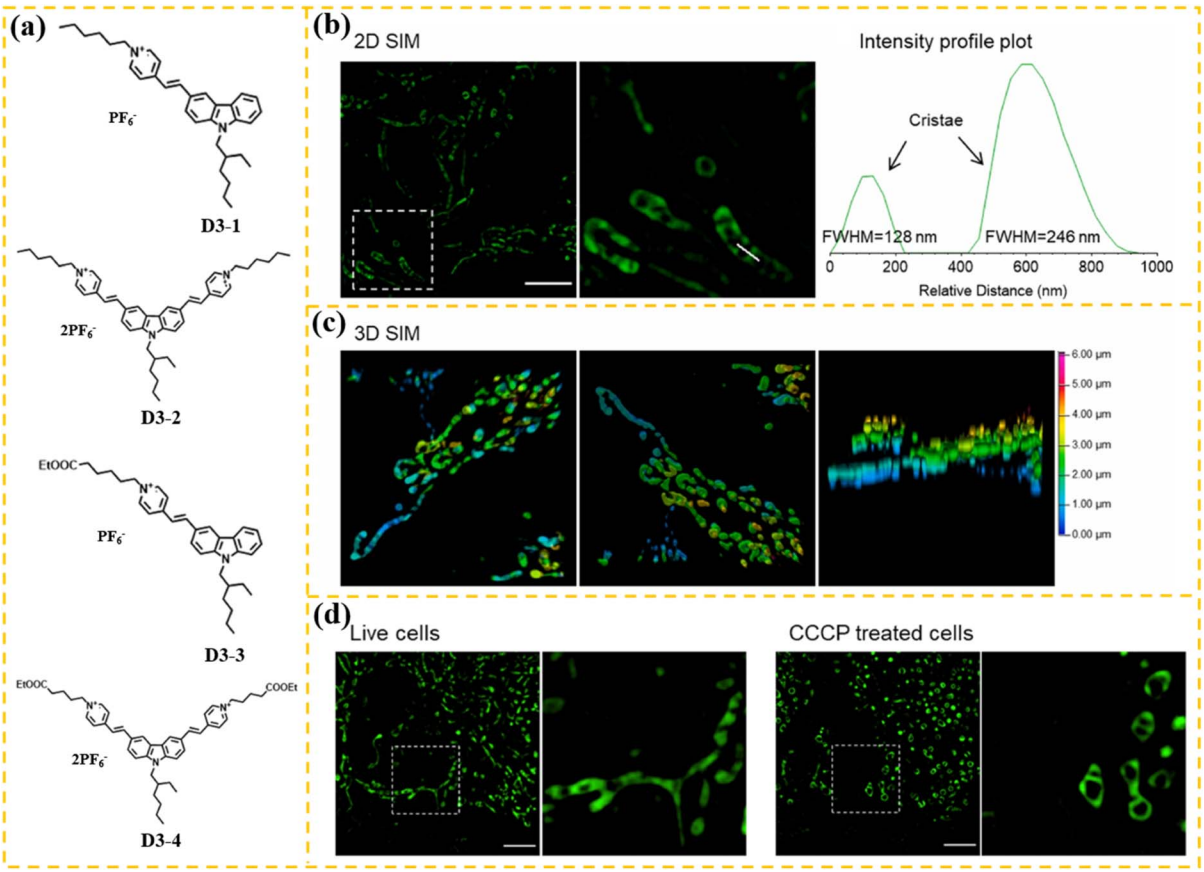

Fig. 6 Super-resolution imaging of probe **D3-*n*** in living cells. (a) Chemical structures of probes **D3-1**, **D3-2**, **D3-3**, and **D3-4**. (b) and (c) 2D and 3D SIM imaging of probe **D3-1** in mitochondria of live HeLa cells. (d) SIM imaging of the ultrastructure of mitochondrial cristae in HeLa cells under CCCP conditions. Content adapted with permission from Elsevier, copyright 2023, as cited in ref. 56.

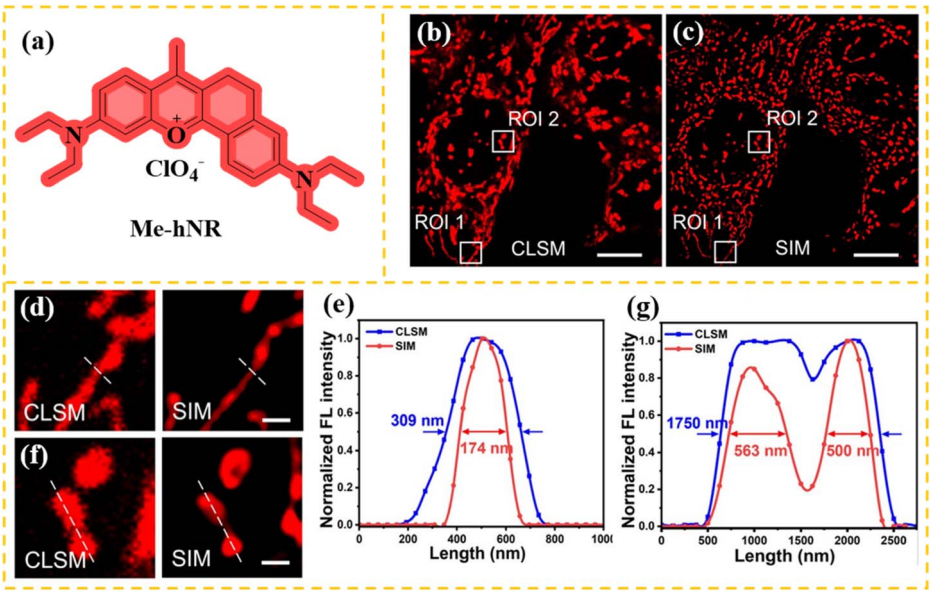

Fig. 7 (a) Chemical structure of the **Me-hNR** probe. (b–g) SIM and CLSM imaging of probe **Me-hNR** in MCF-7 cells. Content adapted with permission from the American Chemical Society, copyright 2024, as cited in ref. 57.

energy, as well as a variety of metabolic processes. Through oxidative phosphorylation, mitochondria convert nutrients into ATP (adenosine triphosphate), which the cell requires to provide energy for various cellular activities.[39] In addition, mitochondria are involved in a variety of cellular functions such as signaling, cell death, and endocrine regulation.[40,41] This organelle with a double-membrane structure contains unique genetic material, mitochondrial DNA (mtDNA) and RNA, and its independent genetic system implies that mitochondria may have originated from the endosymbiotic evolution of ancient symbiotic bacteria. Abnormal mitochondrial function is closely linked to various diseases, including neurodegenerative and metabolic diseases.[42,43] In view of this, mitochondrial biology research has become an important breakthrough in the field of modern life sciences and clinical medicine, and its research results have far-reaching significance in revealing the pathogenesis of diseases and developing novel therapeutic strategies.

Fluorescent probes for mitochondrial SIM imaging are designed with specific fluorescent molecules or dyes that can have an affinity and bind to the mitochondrial outer membrane, the inner membrane, or other specific mitochondrial structures. By specifically labeling mitochondria, these probes help researchers perform clear imaging under super-resolution microscopy, which in turn reveals mitochondrial morphology, dynamic changes, and function. As shown in Fig. 4, common mitochondrial targeting units include quaternized pyridine molecules, triphenylphosphine (TPP), indole, cyanine, pyridine, and rhodamine.[44–46] Common mitochondria-targeted fluorescent probes include dyes based on lipid-soluble or ionic properties, which penetrate the cell membrane and are enriched in mitochondria.[47–52] In addition, these fluorescent probes are capable of providing high-contrast and high-resolution images in SIM imaging by combining them with structured light modes to enhance the imaging details of mitochondria, facilitating the observation of dynamic mitochondrial processes such as changes in membrane potential, fission-fusion events, or metabolic activities.[53,54] These probes are widely used in cell biology, neuroscience and disease mechanism research, and provide key technical support for analysing the association between mitochondrial dysfunction and human diseases.

In 2021, Xu *et al.* developed a SNAP-labelled 4-azetidinyl-naphthamide-derived fluorescent probe **AN-BG**,[55] for long-

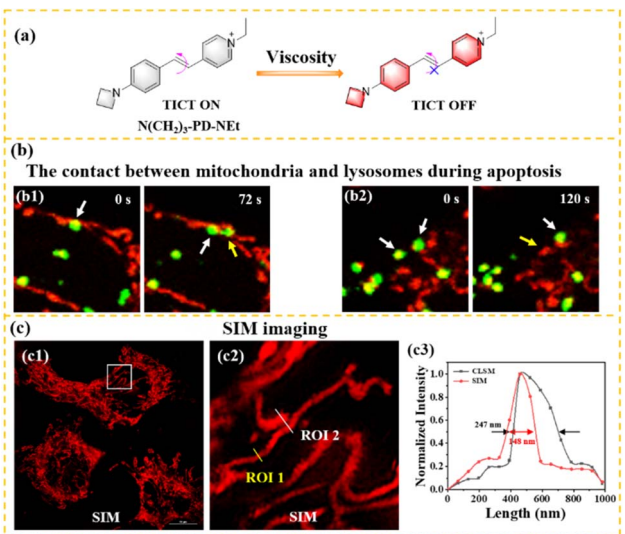

Fig. 8 (a) Response mechanism of probe $N(CH_2)_3$-PD-NEt with viscosity. (b) Imaging of probe $N(CH_2)_3$-PD-NEt of the interaction between mitochondria and lysosomes during apoptosis. (c) SIM imaging of probe $N(CH_2)_3$-PD-NEt in HepG2 cells. Content adapted with permission from the American Chemical Society, copyright 2024, as cited in ref. 58.

duration super-resolution imaging of mitochondrial dynamics. As shown in Fig. 5, long-term super-resolution imaging of mitochondrial dynamics was realized using the probe **AN-BG**, and dynamic mitochondrial fusion and fission processes were also successfully observed. In addition, the probe revealed multiple modes of interaction between mitochondria and lysosomes through two-colour SIM imaging. Overall, this study represents a significant advancement in the use of fluorescent probes for dynamic monitoring of organelles, offering a novel tool for understanding the complex interactions between cellular structures and advancing the application of super-resolution imaging techniques in cellular biology research. This article presents the AN-BG fluorescent probe as a powerful tool for long-term super-resolution imaging of mitochondria, demonstrating its exceptional photostability and chemical stability. However, the complexity of probe synthesis and its associated costs may limit its widespread application, and further investigation is needed to assess its potential nonspecific labelling of non-mitochondrial structures.

In 2023, Tian *et al.* constructed a fluorescent probe **D3-*n*** (ref. 56) with D–π–A and A–π–D–π–A conformations based on *N*-isooctylcarbazole by introducing mono- or pyridine salts. As shown in Fig. 6, this probe exhibits a specific fluorescent response to mitochondrial membranes, selectively targets live cell mitochondria, and can monitor mitochondrial morphological changes in live cells using SIM super-resolution nanoscopy. In addition, the probe successfully revealed the 3D dynamic ultrastructure of mitochondria in living cells under

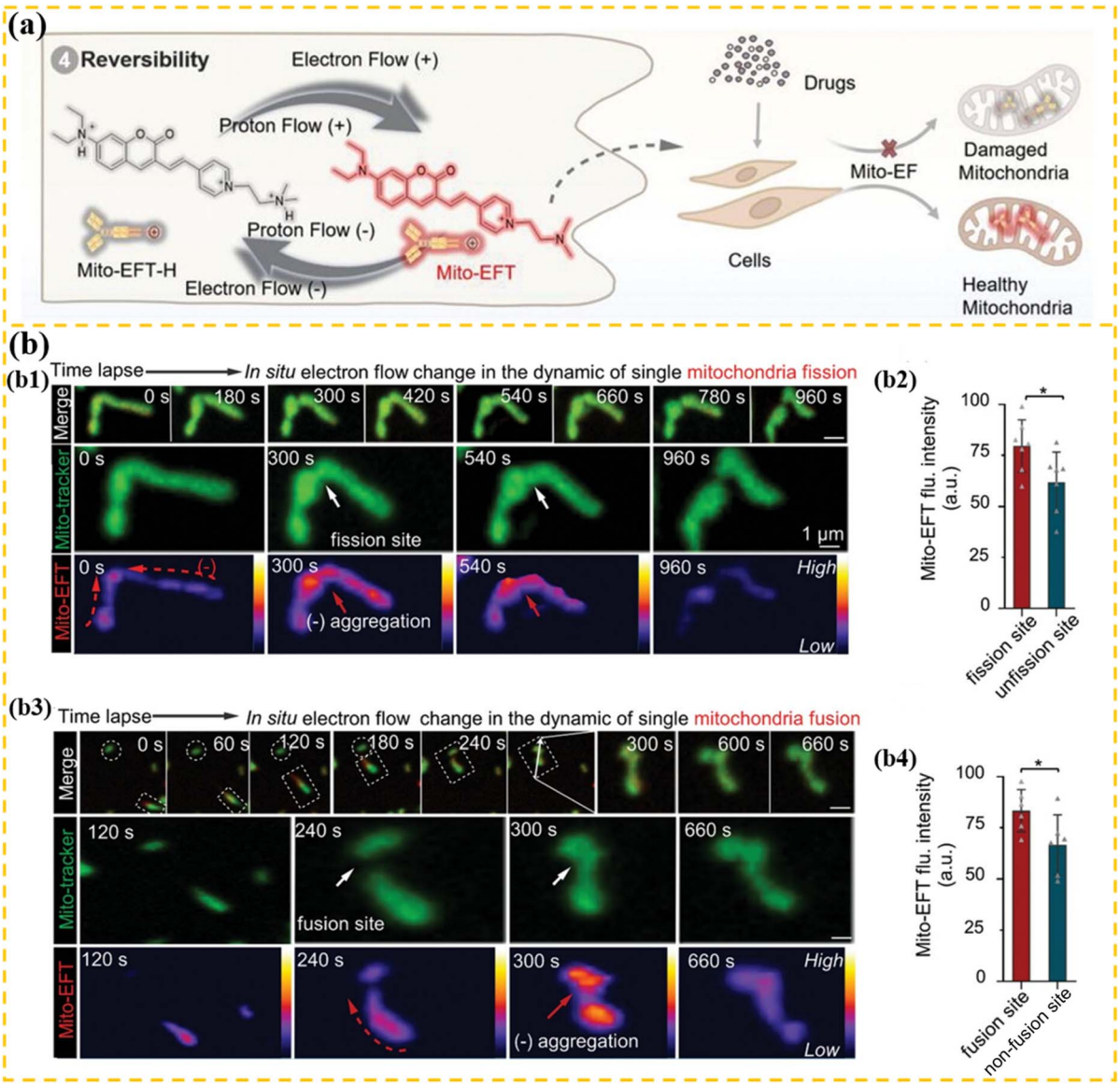

Fig. 9 (a) Schematic diagram of probe **Mito-EFT** in response to mitochondrial electron flow. (b) SIM imaging of the fission and fusion process of probe **Mito-EFT** in HeLa cell mitochondria. (b1 and b2) The fission process of probe **Mito-EFT** in cellular mitochondria and quantitative analysis of probe **Mito-EFT** at the fission site are shown. (b3 and b4) Fusion process of probe **Mito-EFT** in HeLa cell mitochondria and quantitative analysis map of probe **Mito-EFT** at the fusion site. Reproduced with permission from Wiley, copyright 2025, as cited in ref. 59.

oxidative phosphorylation conditions. This article demonstrates the design of a carbazole-based fluorescent probe for super-resolution 3D dynamic imaging of mitochondria, highlighting its high specificity and large Stokes shift. However, the long-term phototoxicity and biocompatibility of the probe in complex biological systems still require further evaluation.

In 2024, Shi *et al.* synthesized an ultra-bright fluorescent probe, **Me-hNR**,[57] based on a xanthene ring for super-resolution SIM imaging of mitochondria. As shown in Fig. 7, the probe has a fluorescence quantum yield of up to 0.92 and excellent photostability, and is capable of rapidly targeting mitochondria at ultra-low concentrations (5 nM). In addition, the probe **Me-hNR** can be used to visualize the structure of mitochondria in living cells by SIM super-resolution imaging with a spatial resolution of up to 174 nm, which provides a novel super-resolution imaging tool for monitoring subcellular mitochondrial structures in living cells. This study highlights the remarkable potential of the **Me-hNR** fluorescent probe in super-resolution imaging. Through meticulous design and optimization, **Me-hNR** exhibits high fluorescence brightness, stability, and favorable photophysical properties for super-resolution imaging. It not only offers a novel tool for observing mitochondrial structural changes but also provides significant support for research on related diseases. However, its universality across different cell types and long-term imaging performance still require further validation. Additionally, simplifying the synthesis method and improving cost-effectiveness are key areas for future optimization.

In the same year, our group also developed a fluorescent probe **N(CH$_2$)$_3$-PD-NEt** targeting mitochondria for dynamic fluorescence imaging of mitochondria in living cells.[58] As shown in Fig. 8, the fluorescence change process of mitochondria during apoptosis was explored using this probe, and the process of mitochondria–lysosome interaction was also observed. In addition, the probe was able to be successfully used for SIM super-resolution imaging. The **N(CH$_2$)$_3$-PD-NEt** fluorescent probe proposed in this article offers a new tool for studying the interaction between mitochondria and lysosomes during apoptosis, demonstrating its potential for super-resolution imaging. However, the probe's specificity and long-term stability in complex biological environments require further validation, and its potential interference with non-target organelles needs to be thoroughly investigated.

In 2025, Chen's group constructed a four-pronged fluorescent probe, **Mito-EFT**, based on coumarin and pyridine moieties for SIM imaging.[59] As shown in Fig. 9, the probe was able to be encapsulated in nanoparticles, giving it full access to the cell. Through SIM imaging, the authors used the probe **Mito-EFT** to visualize and track mitochondrial electron flow (**Mito-EF**) activity within individual mitochondria, providing insights

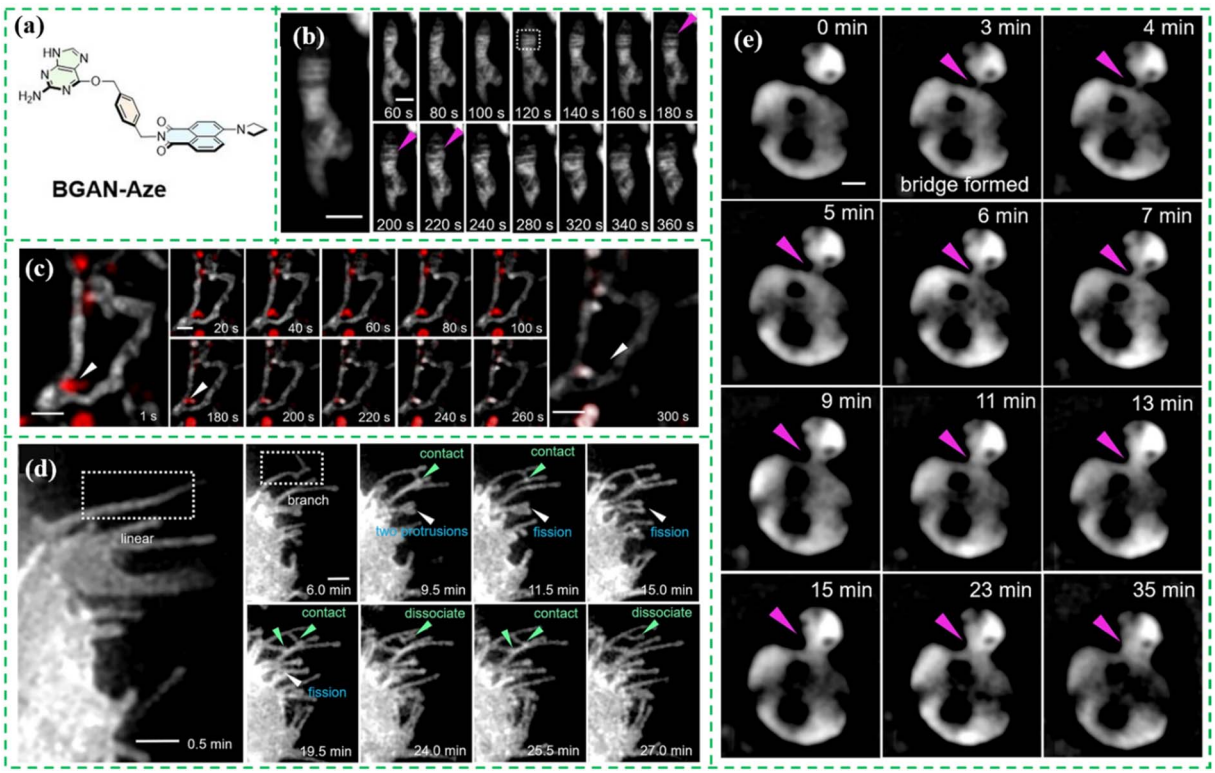

Fig. 10 Super-resolution imaging of the probe **BGAN-Aze** in live HeLa cells. (a) Schematic representation of the chemical structure of the **BGAN-Aze** probe. (b) SIM imaging of mitochondria and mitochondrial cristae labelled with the **BGAN-Aze** probe. (c) Interaction between mitochondria labelled by the **BGAN-Aze** probe and lysosomes. (d) SIM imaging of the cell membrane fusion and fission process of the SNAP-tag-ADRβ2 fusion protein labelled by the **BGAN-Aze** probe. (e) SIM imaging of the nucleolus fusion process in cells, labelled by the **BGAN-Aze** probe. Reprinted with permission from Wiley, copyright 2022, as cited in ref. 60.

beyond traditional morphological assessment methods. In addition, the dynamic processes of **Mito-EF** in mitochondria were successfully revealed using this probe. Furthermore, the accuracy of **Mito-EFT** in detecting **Mito-EF** and evaluating mitochondrial quality was derived from different drug screening methods. This article makes significant progress in the development of novel mitochondrial electron flow imaging probes, providing a new tool for mitochondrial function and drug screening studies. However, the emission wavelength of **Mito-EFT** limits its potential for *in vivo* applications, and its long-term stability and specificity in complex biological systems still require further validation. Additionally, while the article demonstrates the use of **Mito-EFT** in drug screening, a more in-depth investigation into its response mechanisms to different drug types and screening efficiency is needed.

In 2022, Xu *et al.* synthesized a novel dynamic aggregation-mediated SNAP-tagged fluorescent probe, **BGAN-Aze**.[60] As shown in Fig. 10, the probe was able to precisely target and bind to a variety of SNAP-tagged fusion proteins, achieving a significant enhancement of fluorescence intensity up to 41-fold.[56] The unique feature of **BGAN-Aze** is the delicate balance between non-fluorescent aggregates/dimers (A–D) and fluorescent monomers (M), a mechanism that effectively cuts down on fluorescence background noise, thus enabling **BGAN-Aze** to image proteins inside and outside the cell at super-resolution without a washing step. With **BGAN-Aze**, the team has successfully tracked a variety of biological dynamics such as mitochondria–mitochondria contact, mitochondrial autophagy, nucleolus fusion, and filamentous pseudopod growth and contact. The **BGAN-Aze** probe significantly enhances the

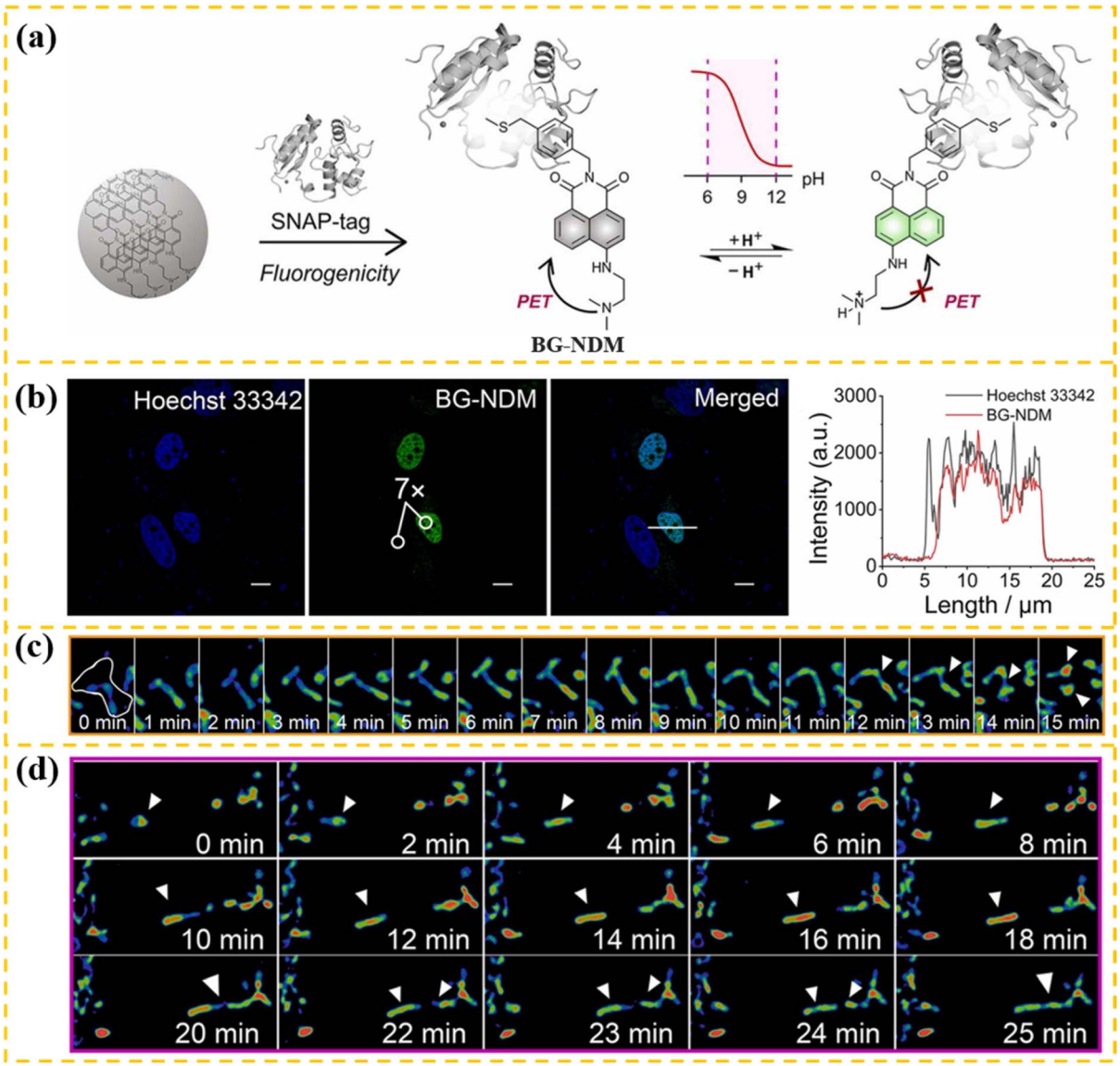

Fig. 11 Super-resolution imaging of pH detection in live cells using the **BG-NDM** probe. (a) Chemical structure of the **BG-NDM** probe and its pH sensing mechanism. (b) Confocal imaging without the washing step was conducted on SNAP-tagged proteins that were marked using the **BG-NDM** probe. (c) and (d) Super-resolution imaging of pH dynamics in mitochondria within HeLa cells using the **BG-NDM** probe. Reproduced with permission from Elsevier, copyright 2024, as cited in ref. 61.

specificity and sensitivity of fluorescence imaging through a dynamic aggregation-regulation mechanism, providing a powerful tool for long-term super-resolution imaging. However, the conversion efficiency between its aggregated and monomeric states, as well as its stability in different cellular environments, still requires further optimization to ensure reliable imaging across a broader range of biological systems. Additionally, while the article demonstrates the application of **BGAN-Aze** in various organelles and biological processes, its long-term phototoxicity and biocompatibility in complex biological samples need to be systematically evaluated.

In 2024, the research team developed a pH-sensitive probe **BG-NDM**, utilizing a light-induced electron transfer mechanism for super-resolution imaging of subcellular pH dynamics.[51] As illustrated in Fig. 11, using the SNAP-tag protein labelling technology, the **BG-NDM** probe allows precise detection of pH levels near target proteins. The aggregate/monomer equilibrium enables wash-free imaging of **BG-NDM** in live cells. Furthermore, through SIM imaging, they successfully captured the temporal variations in pH levels in the vicinity of the mitochondrial protein COX8A during mitochondrial contact, fusion, and fission processes. The BG-NDM probe offers a powerful new tool for super-resolution imaging of subcellular pH dynamics, with its broad pH response range and wash-free imaging properties significantly enhancing experimental convenience and accuracy. However, despite its excellent performance in alkaline environments, the stability and response sensitivity of the probe under extreme acidic conditions still require further validation. Additionally, the probe's versatility across different cell types and subcellular structures, as well as potential photobleaching and biocompatibility issues during long-term imaging, remain important areas for future research.

Tumorigenesis is closely linked to DNA damage and mitochondrial swelling, offering important research directions for the treatment of cancer and other diseases. In 2021, Mao *et al.* investigated a fluorescent probe, **MitoMN**,[62] which continuously labels both mitochondrial lipids and mtDNA for super-resolution imaging of mitochondrial dynamics. As illustrated in Fig. 12, the **MitoMN** probe can induce mitochondrial swelling and cell death in cancer cells upon light activation. Using structured illumination microscopy (SIM), the probe successfully elucidated the interaction dynamics and fusion

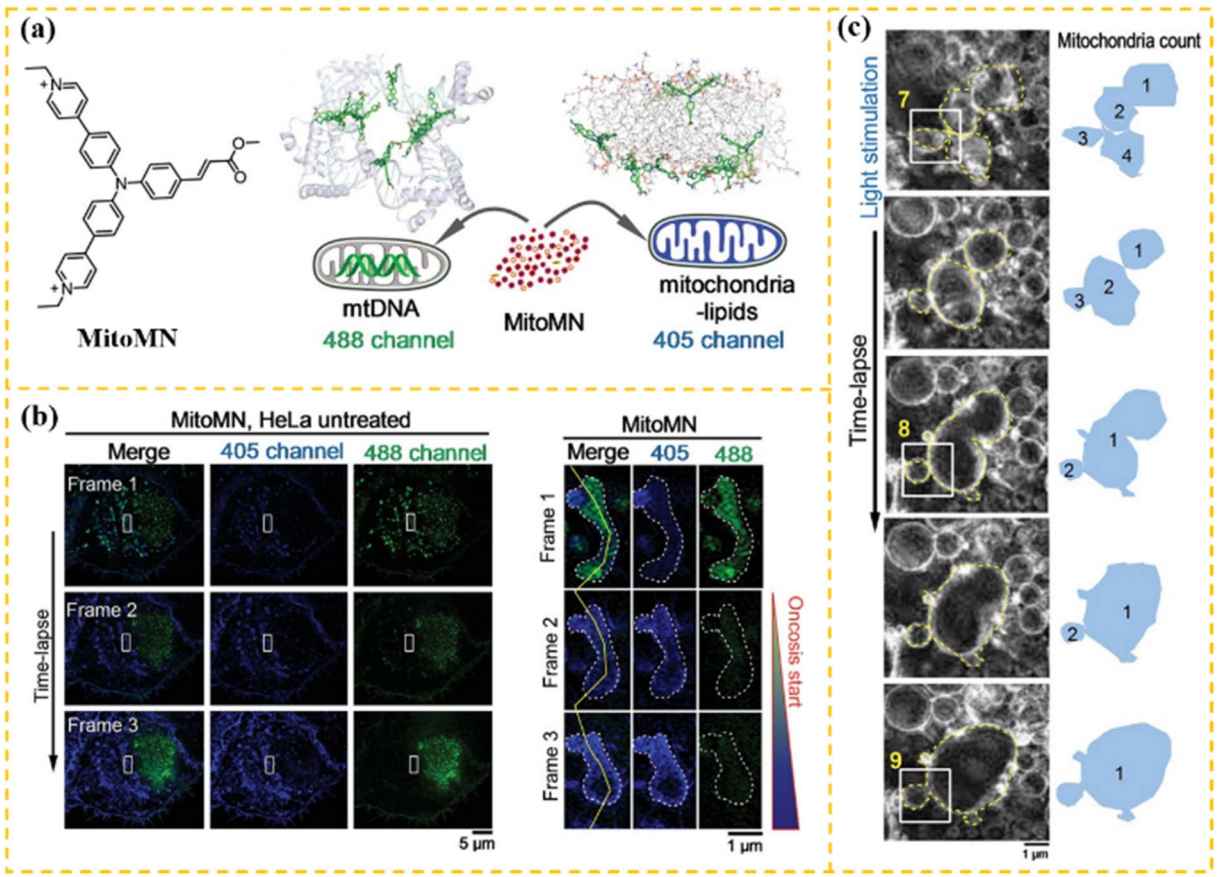

Fig. 12 (a) The molecular architecture of the probe **MitoMN** and simulation of probe **MitoMN** targeting mtDNA and mitochondrial lipids under 405 nm and 488 nm SIM lasers. (b) Colour change of probe **MitoMN** induced by 405 nm and 488 nm SIM laser stimulation and imaging of the mitochondrial swelling process. (c) SIM imaging of the nonlinear mitochondrial enlargement process of probe **MitoMN** in tumours. Reproduced with permission from Wiley, copyright 2021, as cited in ref. 62.

events occurring within swollen mitochondria. Additionally, the probe recorded that when mitochondria exhibit individual enlargement, multiple enlarged mitochondria can interact and fuse into a single large mitochondrion under light stimulation, leading to further nonlinear swelling. The occurrence of this event could serve as a unique, irreversible marker of carcinogenesis, potentially driving a series of novel investigations into the biological intricacies of tumours. In summary, the design of the **MitoMN** probe provides an innovative tool for studying mitochondrial dynamics and cell death mechanisms, with its dual-colour imaging capability and ability to reveal mitochondrial nonlinear expansion phenomena offering significant scientific value. However, the article mainly focuses on observations at the mitochondrial level, with limited discussion on the impact of light activation-induced necroptosis on other organelles and molecular mechanisms. Future research should further optimize the probe properties to expand its application scope and deepen the exploration of the molecular mechanisms of light activation-induced cell necroptosis.

In 2024, in order to further investigate the intricate dynamics associated with the mitochondrial membrane and mtDNA, the team constructed a dual colour fluorescent probe **mtGLP**, featuring a D–π–A structural design for monitoring the dynamic processes of the mitochondrial membrane and mtDNA.[63] As shown in Fig. 13, the author applied the probe **mtGLP** and studied the response kinetics of mtDNA to mitochondrial damage through SIM imaging technology, observing the formation of mitochondrial nucleoid condensation. In addition, it was observed that the peripheral fission of cell nuclear aggregates and mitochondria is related to oxidative stress, and simulations revealed that nuclear aggregates drive peripheral fission through high membrane curvature. This study helps to reveal the steady-state mechanism of non-functional mtDNA degradation, particularly the relationship between the

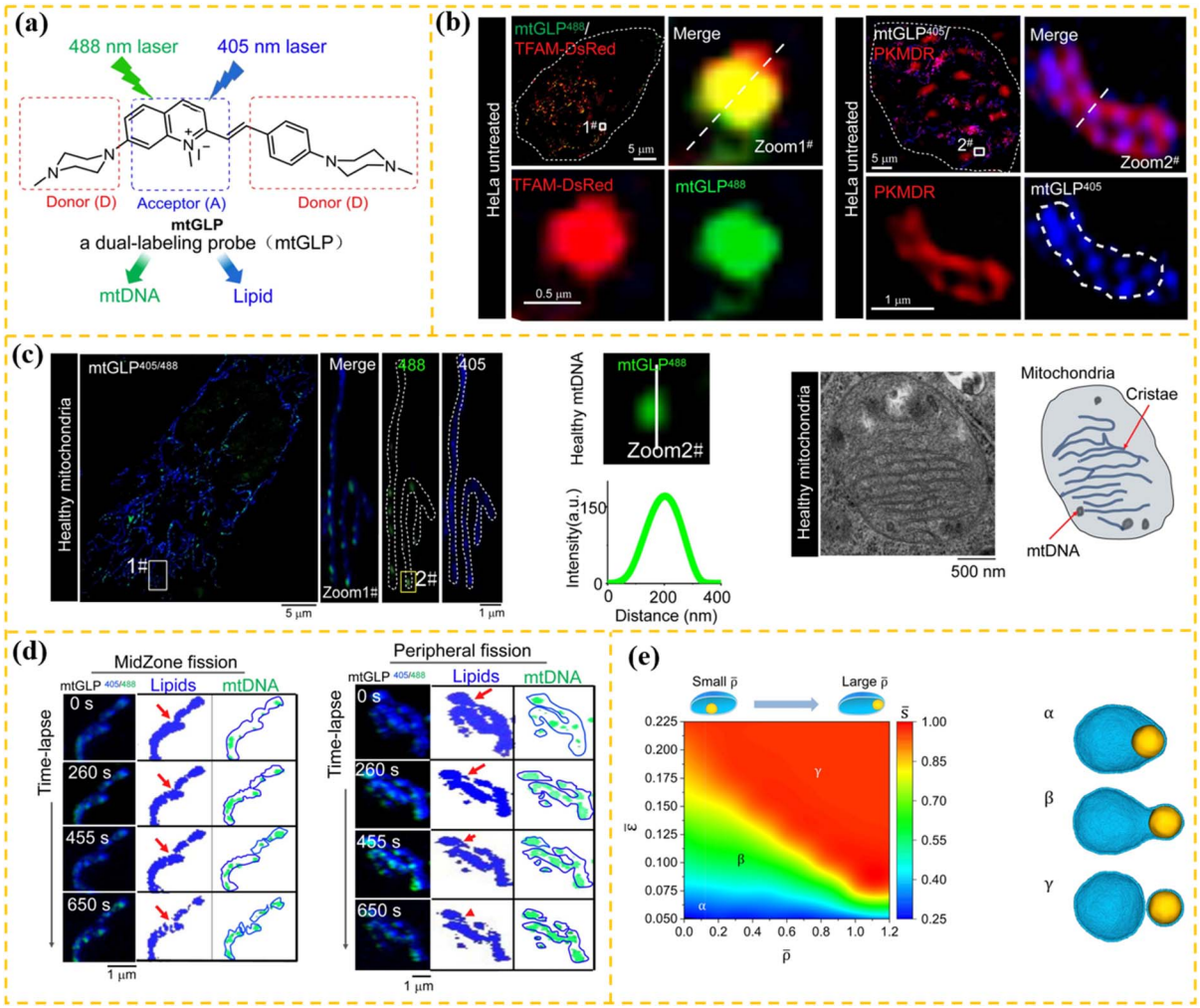

Fig. 13 Super-resolution imaging of the **mtGLP** probe simultaneously labeling the mitochondrial membrane and mtDNA in live cells. (a) Diagram illustrating the chemical architecture of probe **mtGLP**. (b) Dual SIM visualization of mtDNA and the mitochondrial membrane in live cells using the probe **mtGLP**. (c) Dynamics imaging of the **mtGLP** probe in the process of mitochondrial damage in cells. (d) SIM imaging of the **mtGLP** probe in the dynamic process of mitochondrial fission and peripheral division under oxidative stress in HeLa cells. (e) The membrane morphology of mtDNA condensates and membranes exhibits varying strengths and spontaneous curvatures. Reproduced with permission from Elsevier, copyright 2023, as cited in ref. 63.

formation of nuclear-like condensates and asymmetric separation of mitochondrial peripheral fission. Overall, the development of the **mtGLP** probe provides a powerful tool for studying mitochondrial dynamics and mtDNA organization, with its dual-labeling and super-resolution imaging capabilities significantly enhancing our understanding of mitochondrial behaviour. However, while the article reveals the relationship between nucleotide condensates and mitochondrial peripheral fission, the molecular mechanisms underlying this process still require further investigation.

In the same year, the team developed a platinum(II) complex fluorescent probe **Pt1**, based on the excellent DNA-binding ability and selectivity of triphenylamine, to study the dynamic processes of mitochondrial DNA (mtDNA).[64] As shown in Fig. 14, SIM imaging revealed that the probe could induce two distinct aggregation patterns of mtDNA. Over time, **Pt1**-labeled mitochondria gradually aggregated, expanded, and then escaped from the mitochondria, ultimately fusing with lysosomes. The authors also explored the mitochondrial autophagy process triggered by the **Pt1** probe in the MDV pathway of cells. As an effective tool for investigating mitochondrial microautophagy, this probe exhibits significant potential for utilization in cellular imaging and disease-related research endeavours. However, the precise molecular mechanisms underlying **Pt1**-induced mtDNA aggregation and mitophagy remain unclear. Future research could further investigate its action pathways and interactions with other mitochondrial quality control processes.

In 2021, Tan *et al.* utilized the lipophilic cationic triphenylphosphine (TPP) as a mitochondrial targeting group to design and synthesize a series of mtDNA G4-specific fluorescent probes, **MitoISCH** and **MitoPDS**, for monitoring the dynamics of mtDNA G4.[65] Using SIM imaging technology, the study investigated the dynamics of mtDNA G4 in live cells with the **MitoISCH** probe, confirming the widespread folding and generation of mtDNA G4 configurations in cancer cells (as shown in Fig. 15). Additionally, the influence of glycolysis on mtDNA G4 was assessed upon the addition of **MitoPDS**. The results indicated a distinct association between mtDNA G4 folding and glycolytic activity. Finally, the connection between mtDNA G4s and *in vivo* glycolysis was explored. Experimental observations showed that **MitoISCH** accumulated in tumors, with fluorescence intensity peaking when bound to mtDNA G4 in tumor-bearing mice. This study uncovers a novel connection between mtDNA G4s and cellular glycolytic processes, paving the way for the discovery of novel cancer biomarkers and therapeutic targets. Although the studies demonstrate the potential of **MitoISCH** and **MitoPDS** in cellular and animal models, the

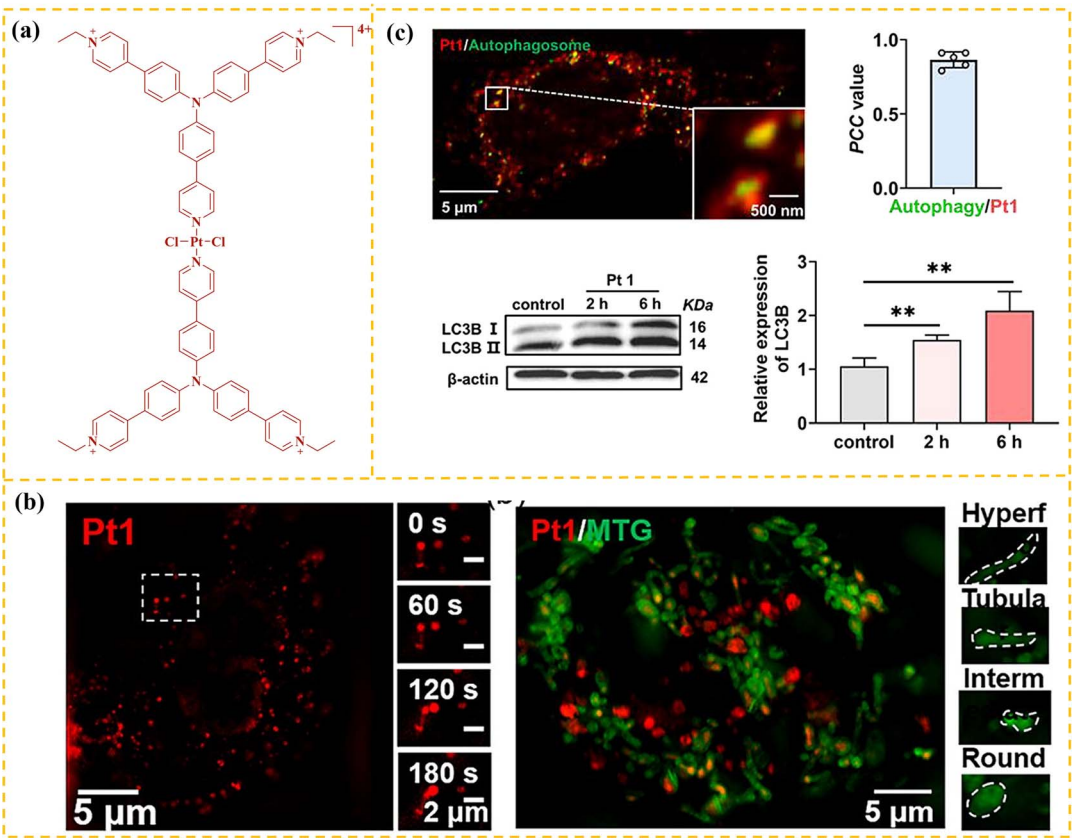

Fig. 14 (a) Chemical structural formula of probe **Pt1**. (b) The fusion and escape process of probe **Pt1** in HeLa cell mitochondria, as well as SIM imaging of mtDNA aggregates escaping from mitochondria. (c) The co-localization imaging of probe **Pt1** and the autophagosome commercial dye DAPRed, as well as the detection process of protein blotting between probe **Pt1** and LC3 in HeLa cells. Reprinted with permission from the American Chemical Society, copyright 2024, as cited in ref. 64.

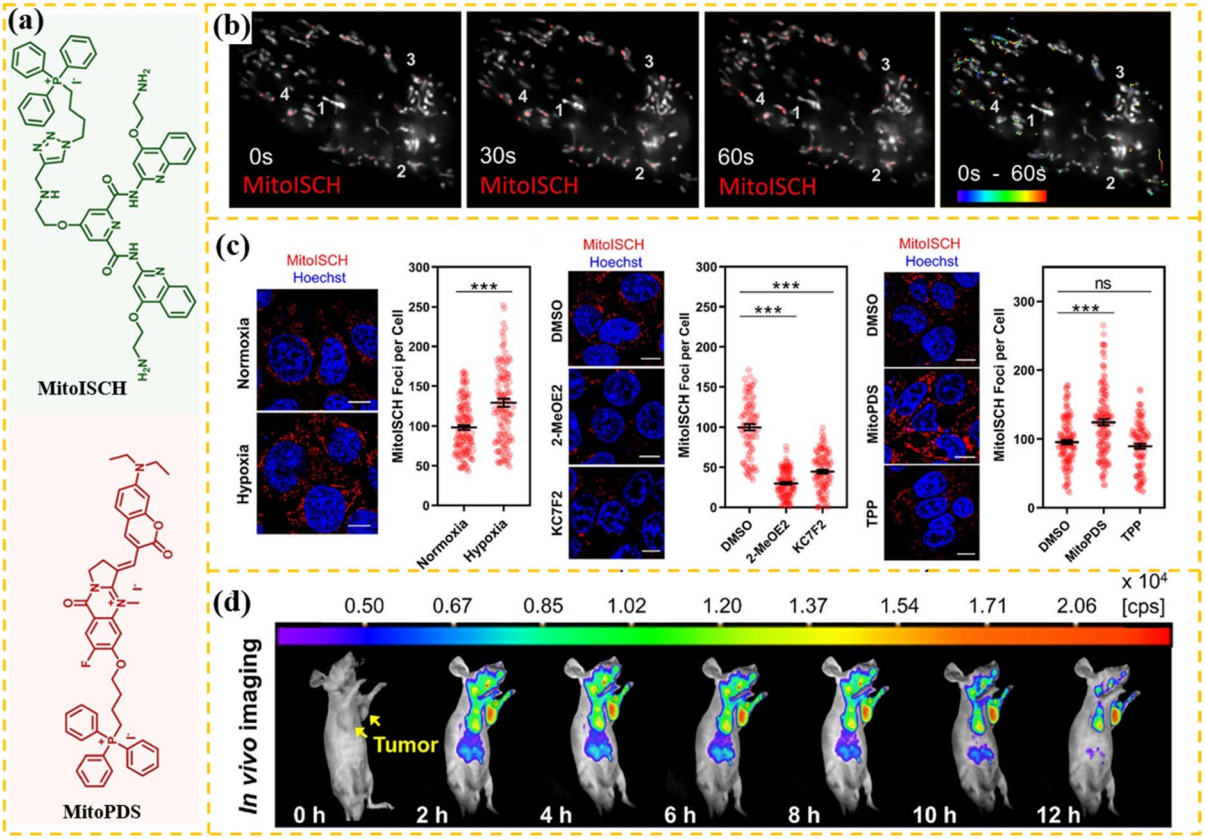

Fig. 15 (a) Chemical structures of the mtDNA G4-specific fluorescent probe **MitoISCH** and the mitochondria-targeted G4 ligand, **MitoPDS**. (b) SIM imaging of mtDNA G4 kinetics of probe **MitoISCH** in living cells. (c) Imaging of mtDNA G4 folding to glycolysis in HepG2 cells under hypoxic stimulation. (d) Imaging of probe **MitoISCH** in glycolysis in hormonal nude mice. Reprinted with permission from the American Chemical Society, copyright 2021, as cited in ref. 65.

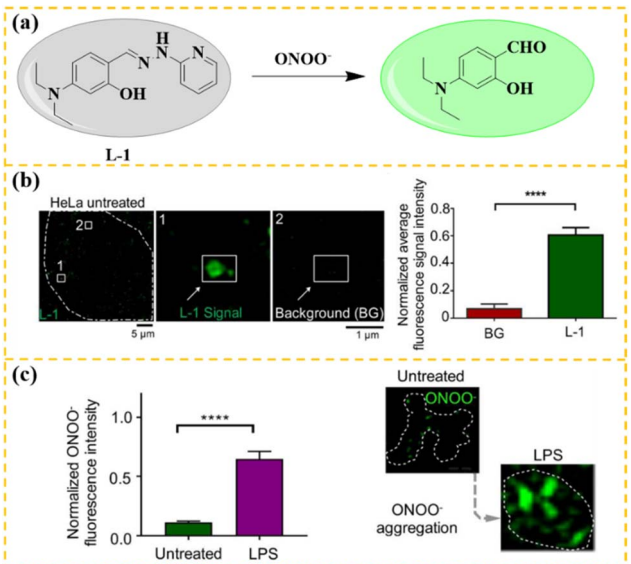

Fig. 16 (a) Response mechanism of probe **L-1** with $ONOO^-$. (b) Super-resolution imaging of $ONOO^-$ generation by probe **L-1** in living cells. (c) Probe **L-1** was used to image the aggregation of $ONOO^-$ during LPS-induced mitochondrial damage. Reproduced with permission from Elsevier, copyright 2021, as cited in ref. 71.

precise mechanisms by which mtDNA G4 structures influence cellular physiological and pathological processes, as well as how **MitoPDS** precisely regulates these structures to affect cellular metabolism, still require further investigation.

3.1.1.1 Mitochondria-targeted fluorescent probes for SIM imaging of biomarkers. Mitochondria, as essential energy factories of the cell, play crucial roles in cellular metabolism, signal transduction, and regulation.[66] During normal physiological functions, mitochondria produce reactive oxygen species (ROS). Although ROS have physiological functions in cellular metabolism, their excessive accumulation can trigger oxidative stress, leading to cellular damage and the onset of various diseases.[67,68] At the same time, reactive sulfur species, such as hydrogen sulfide, have attracted growing interest in recent times due to their role in regulating mitochondrial function and antioxidant stress responses.[69,70] Traditional observation methods often fail to provide high-resolution, real-time dynamic imaging to explore the complex interactions between mitochondria, ROS, and reactive sulfur. The advent of structured illumination microscopy (SIM) has overcome this limitation, offering exceptional spatial resolution to visualize dynamic changes in mitochondria and their interactions with ROS and reactive sulfur. This technology provides powerful support for studying

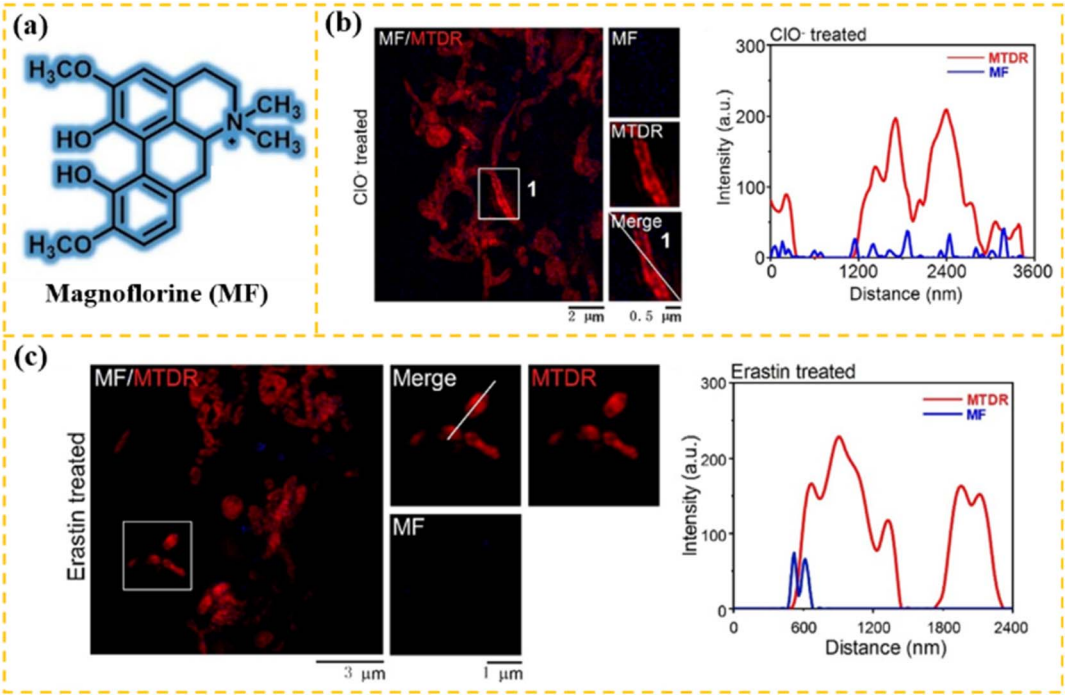

Fig. 17 (a) The molecular structure of the pharmaceutical compound **MF**. (b) Visualized SIM imaging of the drug molecule **MF** detecting cellular mitochondrial ClO^-. (c) **MF** imaging of the mitochondrial change process during erastin-induced iron death. Reproduced with permission from Elsevier, copyright 2022, as cited in ref. 72.

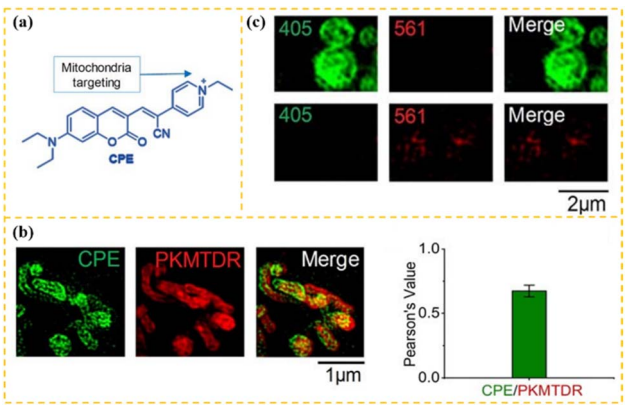

Fig. 18 (a) Chemical structure of probe **CPE**. (b) Co-localized SIM imaging of probe **CPE** and commercial dye PKMTDR in HeLa cells. (c) CCCP-induced probe **CPE** for detection of RSS in HeLa cells. Reproduced with permission from Frontiers, copyright 2022, as cited in ref. 73.

intracellular redox states, mitochondrial function, and the mechanisms of related diseases.

In 2021, Wang *et al.* designed and synthesized a "landmine" probe **L-1** based on 4-(*N*,*N*-diethylamino)salicylaldehyde conjugated dye as the fluorophore, 2-hydrazinopyridine as the $ONOO^-$ recognition receptor, and a C=N bond as the linker for $ONOO^-$ SIM imaging.[71] As shown in Fig. 16, the probe **L-1** exhibited exceptional sensitivity and selectivity towards $ONOO^-$, boasting a detection limit of 85.7 nM. Utilizing probe **L-1**, the generation of $ONOO^-$ in the mitochondrial cristae of the cells was successfully revealed with a resolution of 100 nm, and the process of intracellular $ONOO^-$ changes induced by LPS was also investigated. This work contributes to imaging the

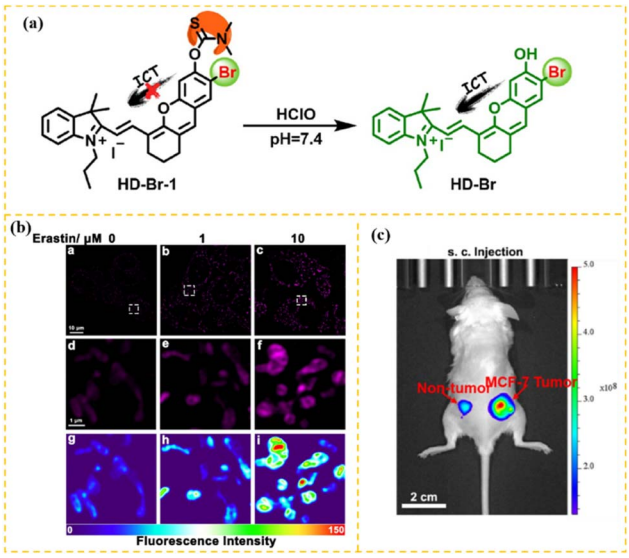

Fig. 19 (a) Chemical structure of probe **HD-Br-1** and the reaction mechanism with HClO. (b) SIM imaging of probe **HD-Br-1** in cells under stimulation at different concentrations of erastin. (c) *In vivo* imaging of probe **HD-Br-1** in tumor and non-tumor regions in mice. Reprinted with permission from the American Chemical Society, copyright 2022, as cited in ref. 74.

detection and monitoring of functionally active small bioactive molecules. However, further studies could explore the application of this probe in more complex biological systems and its potential for *in vivo* imaging.

In the following year, the group screened a natural, unlabeled drug molecule, magnoflorine (**MF**),[72] for monitoring ClO^- in mitochondria by searching a library of herbal compounds. As shown in Fig. 17, compound **MF** has excellent fluorescent properties and can target mitochondria well. The drug molecule **MF** was used for the first time to visualize intracellular mitochondrial ClO^- by SIM imaging. In addition, the authors found that the drug molecule **MF** was able to monitor morphological changes in mitochondria during iron death. This study will be essential for future investigations into the underlying mechanisms of disease processes. This work could pave the way for future research into the role of ClO^- and ferroptosis in various

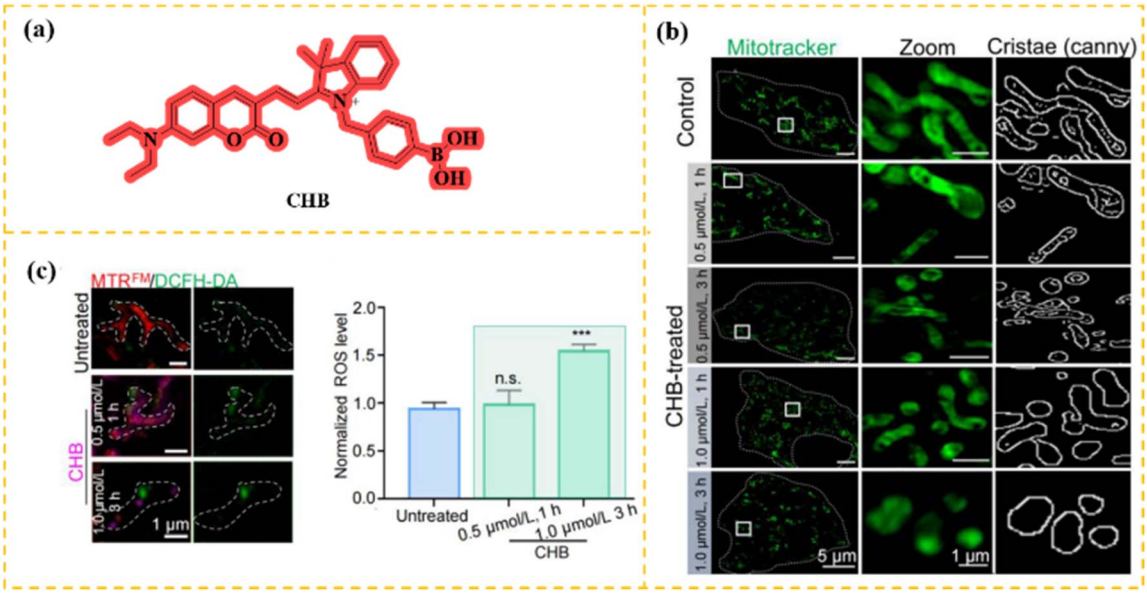

Fig. 20 (a) Chemical structure diagram of probe **CHB**. (b) SIM imaging of the distribution and depletion process of ATP and ADP in cell-tracked mitochondria at different concentrations of probe **CHB** and different incubation times. (c) SIM imaging of mitochondrial morphological damage induced by different concentrations of probe **CHB** at different incubation times. Reprinted with permission from Elsevier, copyright 2024, as cited in ref. 75.

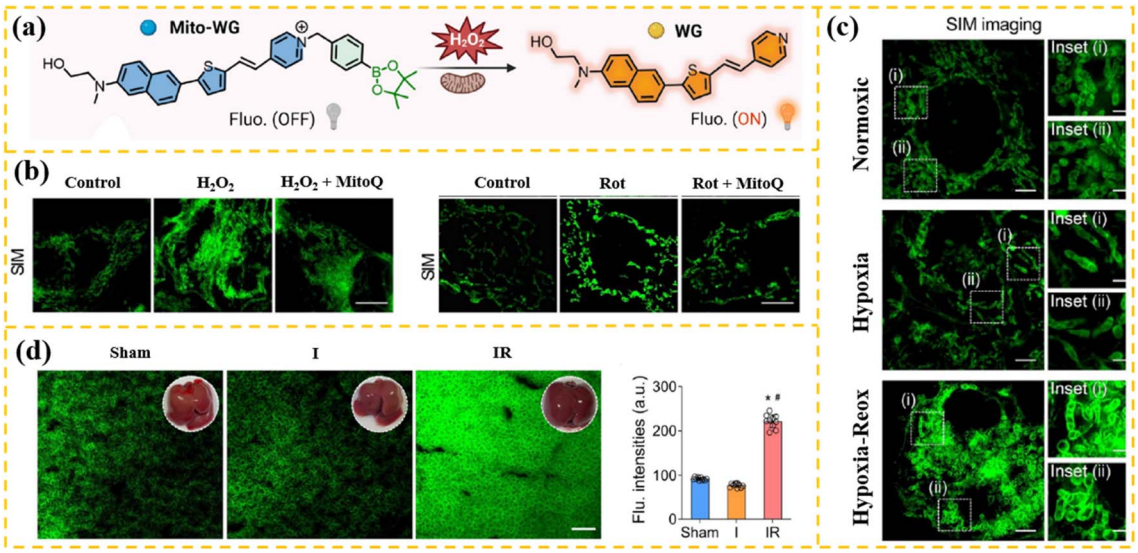

Fig. 21 (a) Response mechanism of probe **Mito-WG** with H_2O_2. (b) SIM imaging of probe **Mito-WG** detecting H_2O_2 in live cells and Rot-stimulated production of mtH_2O_2. (c) SIM imaging of probe **Mito-WG** monitoring mtH_2O_2 during hepatocyte hypoxia-reoxygenation. (d) Probe **Mito-WG** detects fluctuations in the level of mtH_2O_2 in rat liver ischemia-reperfusion injury. Reprinted with permission from Springer Nature, copyright 2024, as cited in ref. 76.

diseases, including neurodegenerative disorders and cancer. However, despite **MF** demonstrating excellent performance in *in vitro* and cellular experiments, the specific molecular mechanisms underlying its interaction with ClO^- and its regulatory role in ferroptosis require further investigation for clarification.

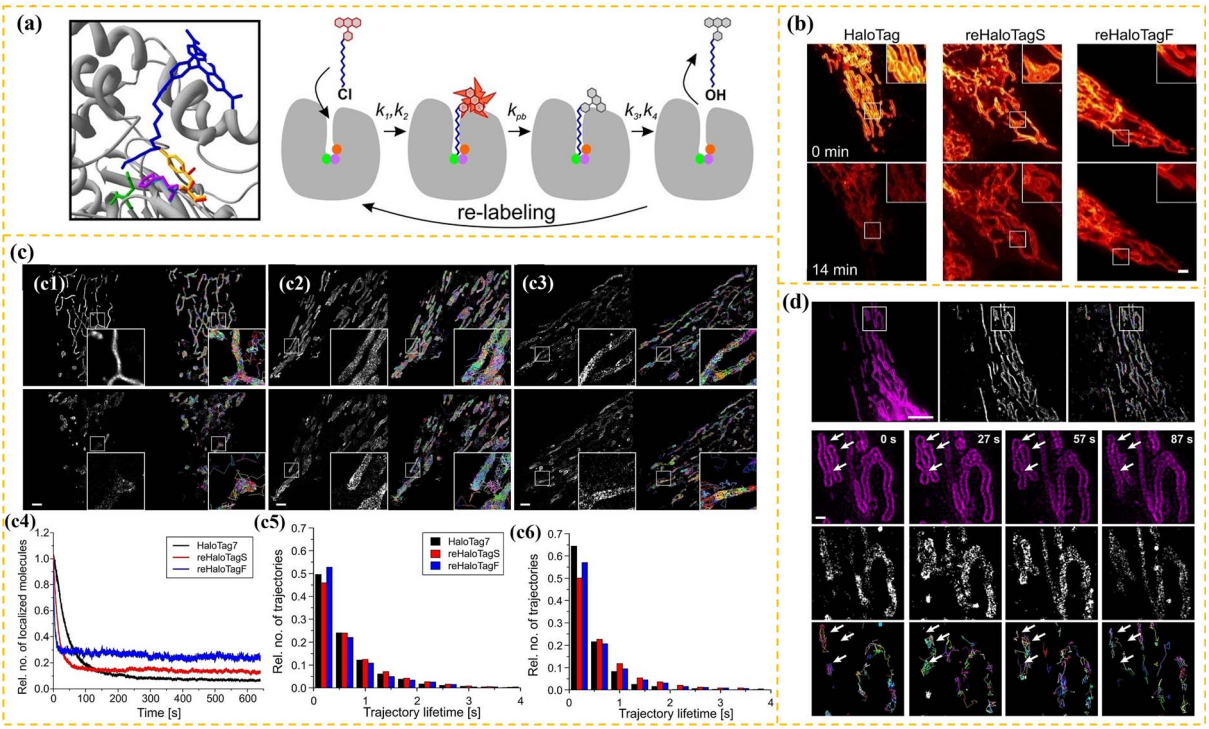

Fig. 22 (a) Generation of **reHaloTagS** and **reHaloTagF** based on HaloTag variants. (b) STED imaging of compounds HaloTag, **reHaloTagS** and **reHaloTagF** in HeLa cells. (c) TALM imaging of compounds HaloTag, **reHaloTagS** and **reHaloTagF** in HeLa cells. (d) Synchronized SIM and TALM imaging of compounds HaloTag, **reHaloTagS** and **reHaloTagF** in HeLa cells. Reprinted with permission from Wiley, copyright 2023, as cited in ref. 77.

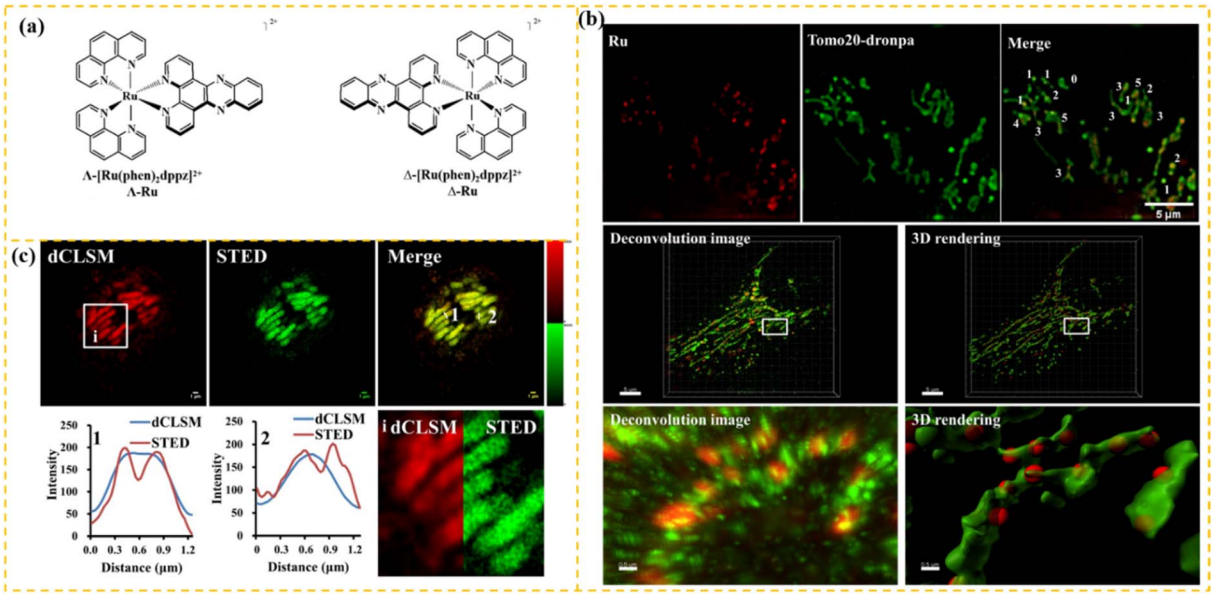

Fig. 23 (a) Enantiomeric structure of compound **[Ru(phen)$_2$dppz] Cl$_2$**. (b) SIM imaging in cellular mitochondria using compound **[Ru(phen)$_2$-dppz] Cl$_2$** with the commercial dye Tomo20-drop. (c) CLSM and STED imaging of **[Ru(phen)$_2$dppz] Cl$_2$** within living cells. Reprinted with permission from Oxford, copyright 2023, as cited in ref. 78.

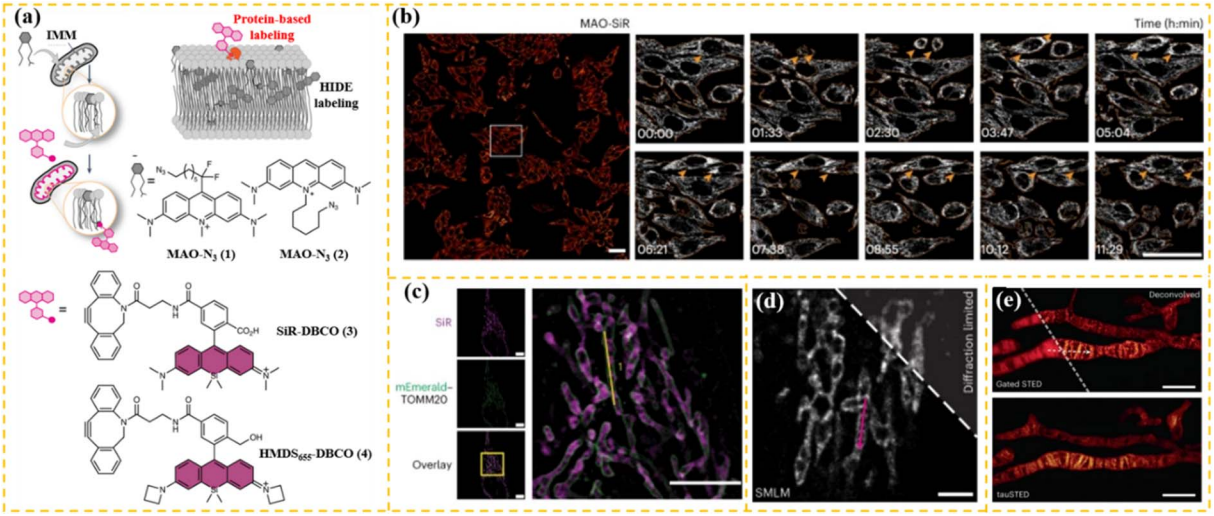

Fig. 24 Novel **HIDE** probes for IMM imaging. (a) Confocal time-lapse imaging of probe **MAO-SiR** in live cells. (b) SIM imaging of probe **SiR-DBCO** with mEmerald-TOMM20 in HeLa cell mitochondria. (c) SMLM imaging of mitochondrial inner membrane cristae by probes **MAO-N_3** and **$HMDS_{655}$-DBCO** in HeLa cells. (d) STED imaging of mitochondrial inner membrane cristae by probe **MAO-SiR** in HeLa cells. Reprinted with permission from Springer Nature, copyright 2024, as cited in ref. 79.

In the same year, Dong *et al.* developed a fluorescent probe **CPE**, specifically designed for detecting RSS in mitochondria using coumarin as a fluorophore and pyridinium salt as an electron-withdrawing group.[73] As shown in Fig. 18, the probe selectively targets mitochondria. Additionally, through SIM imaging, the authors investigated the morphological changes in the **CPE** probe in HeLa cell mitochondria and monitored the variations in reactive sulfur species (RSS) within the mitochondria. They observed an increase in RSS during CCCP-induced mitochondrial damage. This probe offers a design strategy that lays the foundation for accurately measuring mitochondrial morphology and RSS changes in live cells. The results of this study underscore the probe's potential to enhance the understanding of mitochondrial pathophysiology, especially in diseases associated with mitochondrial damage, such as neurodegenerative and metabolic disorders. By enabling real-time visualization and quantification of these alterations in living cells, this research significantly advances the development of tools for investigating cellular stress responses. Future research could explore the wider applications of this probe in various cellular models or *in vivo* systems, aiming to further clarify the role of RSS in disease mechanisms and their possible therapeutic implications.

Next, He *et al.* developed a near-infrared excited/emission fluorescent probe, **HD-Br-1**, based on the *N*,*N*-dimethyldithiocarbamate moiety for selective oxidation-induced cleavage, aimed at rapid detection of HClO within mitochondria.[74] As shown in Fig. 19, using super-resolution SIM imaging, this probe was used to track the fluctuations in mitochondrial HClO levels and morphological changes during ferroptosis in real time. Furthermore, **HD-Br-1** was effectively utilized to track both externally introduced and internally generated hypochlorous acid (HClO) within mitochondria throughout the ferroptotic process, as well as to differentiate between neoplastic and normal tissues. This probe offers a promising tool for studying the ferroptosis mechanisms related to HClO in cancer and exploring therapeutic strategies. Despite the excellent performance of **HD-Br-1** *in vitro* and in cellular assays, its practical application in living organisms remains challenging. Issues such as probe metabolic stability, long-term toxicity, and *in vivo* distribution characteristics require further validation.

In 2024, Chen *et al.* designed and synthesized a fluorescent probe **CHB**, which is based on coumarin and semi-flavone groups, for intracellular detection of mitochondrial ATP and ADP (as shown in Fig. 20).[75] This probe exhibits mitochondrial

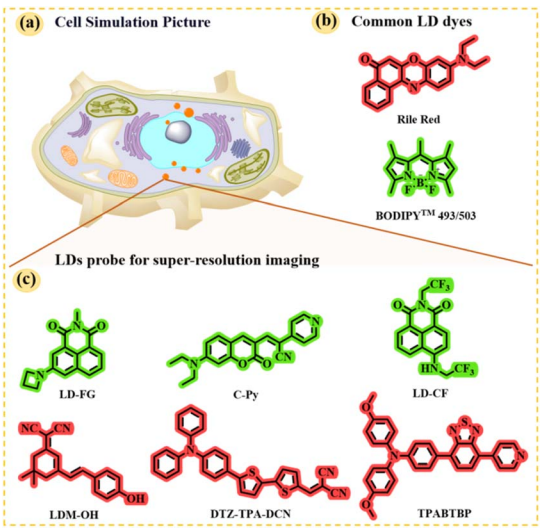

Fig. 25 Structural design of developed lipid droplet-targeted fluorescent probes. (a) Cell simulation diagram. (b) Common lipid droplet dyes Rile Red and BODIPY 493/503. (c) LD probes are capable of being used for super-resolution imaging of lipid droplets.

targeting properties, allowing direct observation of its distribution and activity within mitochondria, and enabling the identification of mitochondrial cristae. Furthermore, under SIM imaging conditions, the authors observed that the depletion of ATP and ADP within cells led to the disruption of mitochondrial membrane potential (MMP) and damage to mitochondrial cristae, ultimately triggering mitochondrial dysfunction. As an effective tool for monitoring the *in situ* mechanism of small-molecule anticancer agents, this probe provides crucial insights for the further discovery of small-molecule drugs. This further elucidates the role of mitochondrial dysfunction in cellular pathological states. This research not only provides a new tool for cancer studies but also offers significant support for investigating the mechanisms of small-molecule drugs. However, the universality of the drug beacon strategy and its potential application in various types of small-molecule drugs warrant further exploration.

In the same year, Li *et al.* designed and synthesized a low-background fluorescent probe **Mito-WG**, using a naphthylamine group functioning as an electron-donating unit, a pyridine cyano group serving as an electron-withdrawing moiety, and a thiophene π-bridge for the connection, aimed at detecting H_2O_2 within mitochondria.[76] As depicted in Fig. 21, the probe displays a TICT (twisted intramolecular charge transfer) effect, resulting in weak fluorescence. Upon entering the mitochondrial matrix, the probe specifically detects H_2O_2. Using super-resolution SIM imaging, the fluctuations in mtH_2O_2 levels under hypoxia/reoxygenation conditions within mitochondria were studied. Additionally, the probe **Mito-WG** was successfully used to reveal the dynamic response of mtH_2O_2 in rat liver ischemia-reperfusion injury, providing strong evidence for the early onset of liver damage, which could lead to better diagnostic and therapeutic strategies. This article offers a new perspective on liver ischemia-reperfusion injury research using the **Mito-WG** probe. However, its clinical translational potential and long-term safety still require further exploration, and the precise mechanisms of mtH_2O_2 need to be further elucidated.

3.1.1.2 Mitochondria-targeted fluorescent probes for multi-modal super-resolution fluorescence imaging. Traditional imaging

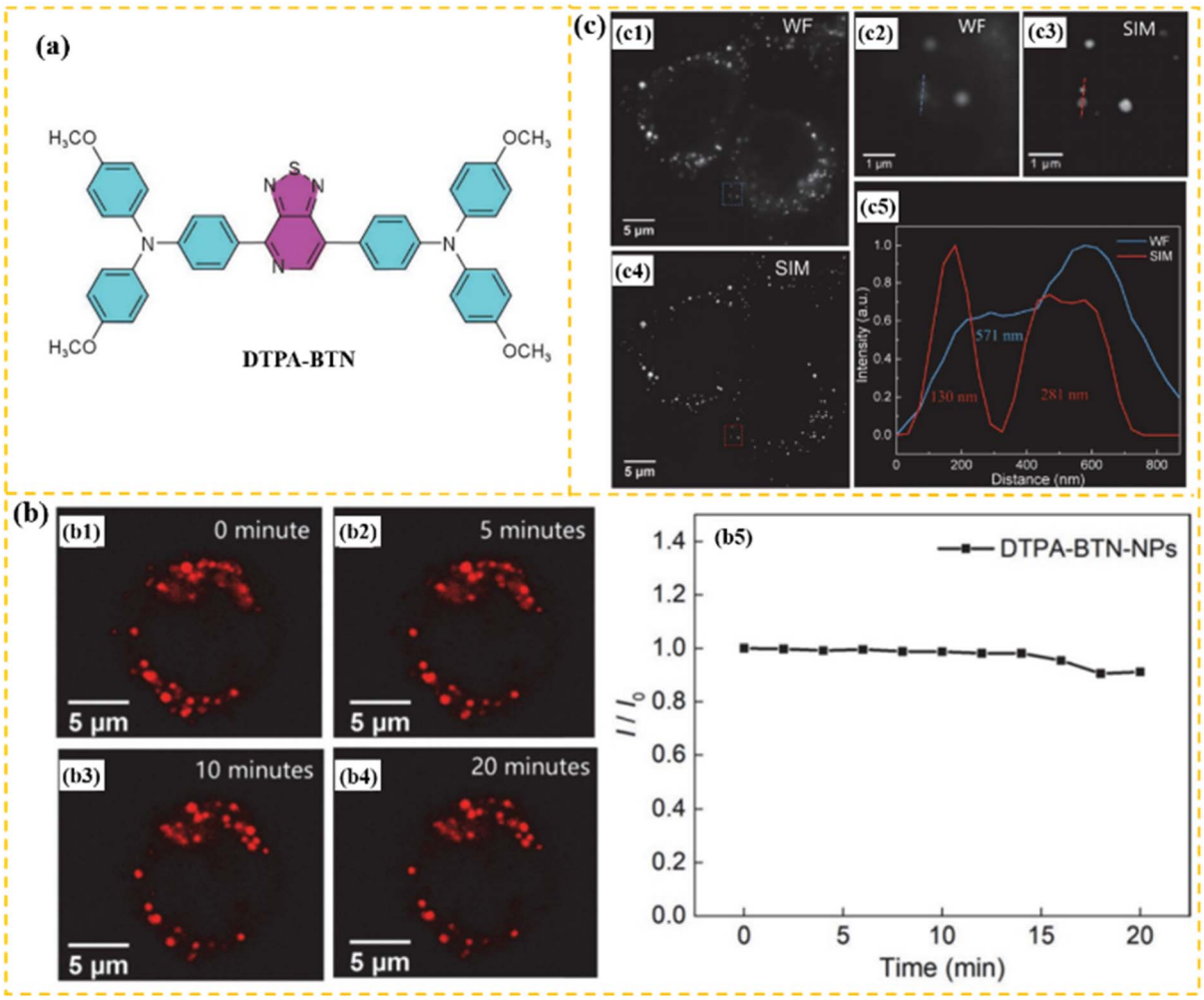

Fig. 26 (a) Chemical structural formula of probe **DTPA-BTN**. (b) Light stability imaging of nanoprobe **DTPA-BTN-NPs** in HeLa cells. (c) Super-resolution SIM imaging of nanoprobe **DTPA-BTN-NPs** in HeLa cells. Reprinted with permission from Springer Nature, copyright 2021, as cited in ref. 83.

techniques struggle to provide sufficient resolution for accurately revealing the microscopic structure and functional alterations within mitochondria. Therefore, the combination of multimodal super-resolution fluorescence imaging techniques and specialized fluorescent probes offers high spatial resolution and real-time dynamic monitoring, thereby serving as a potent instrument for investigating mitochondrial function and its cellular roles. In 2023, Piehler *et al.* synthesized two HaloTag variants, **reHaloTagS** and **reHaloTagF**, based on the reversible labeling of HaloTag7, for multimodal super-resolution fluorescence imaging.[77] As shown in Fig. 22, both reHaloTag variants exhibit excellent photostability and are suitable for various super-resolution fluorescence imaging techniques, such as cLSM, STED, SIM, and TALM. Notably, **reHaloTagF** demonstrates even more remarkable properties. These findings highlight the significance of reHaloTag labeling in advancing the limits of modern fluorescence microscopy technology. This article demonstrates reversible labeling within live cells through the engineered modification of HaloTag, significantly enhancing the long-term imaging capability of fluorescence microscopy. However, its broader application in real biological systems is still limited by potential issues such as probe stability and cytotoxicity, necessitating further optimization.

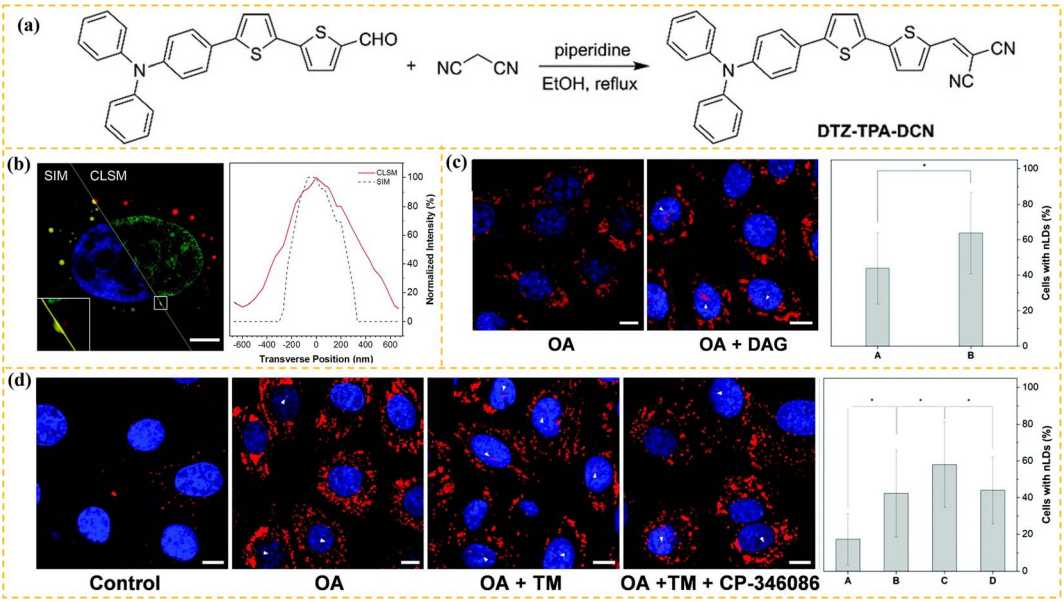

Fig. 27 (a) Synthesis process and chemical structure of probe **DTZ-TPA-DCN**. (b) Confocal and SIM imaging of probe **DTZ-TPA-DCN** in HepG2 cells. (c) Fluorescence imaging of probe **DTZ-TPA-DCN** in DAG-treated cells. (d) Fluorescence imaging of probe **DTZ-TPA-DCN** in oleic acid and clathrin-treated cells. Reprinted with permission from Royal Society of Chemistry, copyright 2021, as cited in ref. 84.

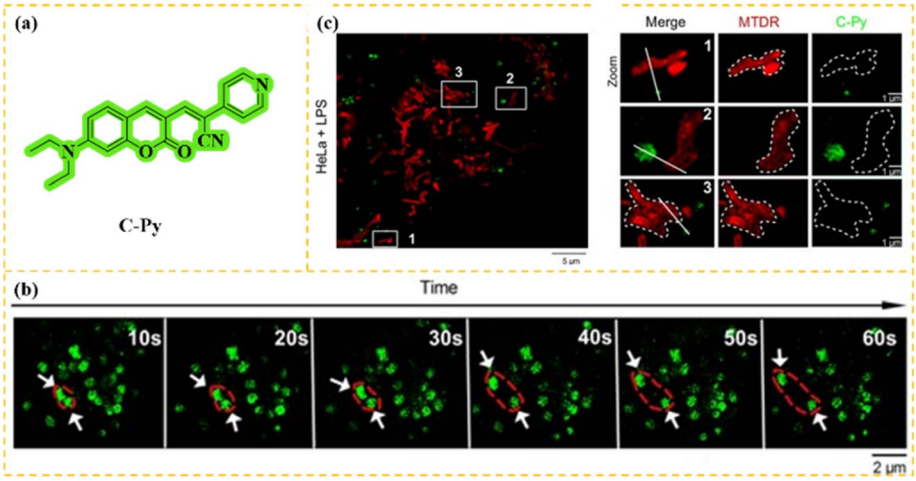

Fig. 28 (a) The molecular architecture of the **C-Py** probe. (b) SIM imaging of probe **C-Py** in HeLa cells over time. (c) Imaging of probe **C-Py** with mitochondrial commercial dye (MTDR) during LPS-induced mutual contact between lipid droplets and mitochondria in HeLa cells. Reprinted with permission from Ivyspring International, copyright 2021, as cited in ref. 85.

During that same year, Zhu and colleagues synthesized a photo-switchable fluorescent probe **[Ru(phen)$_2$dppz] Cl$_2$**, based on chiral ruthenium bipyridine complexes, for specific imaging of mitochondrial DNA within live cells.[78] As depicted in Fig. 23, the authors found that the Δ-enantiomer of **[Ru(phen)$_2$dppz] Cl$_2$** exhibited superior spectral performance in mitochondria compared to the Λ-enantiomer. Additionally, the study observed the migration of the probe from the mitochondria to the nucleus during cellular apoptosis. This research marks the inaugural disclosure of chiral Ru complexes achieving enantioselective targeting and super-resolution imaging of live cell mtDNA *via* the generation and dissociation of ion-pair complexes with appropriate anti-anions. This article achieves super-resolution imaging and photosensitization of mitochondrial DNA using chiral ruthenium–polypyridine complexes, showcasing their potential in biological imaging and photodynamic therapy. However, the specific mechanisms underlying their chiral selectivity and *in vivo* application efficacy require further exploration and validation.

In 2024, Schepartz and colleagues introduced a range of novel environmentally sensitive (**HIDE**) probes assembled from the lipid-like molecule **MAO-N$_3$**. These probes exhibit photostability and non-toxicity, and support ultra-high spatial resolution imaging of mitochondria across multiple modalities.[79] As shown in Fig. 24, the **MAO-SiR** probe enables long-term confocal imaging, demonstrating superior photostability to the commercial mitochondrial dye MitoTracker Deep Red. In SIM imaging, **MAO-SiR** outperforms commercial dyes such as mEmerald-TOMM20 and MitoTracker Green in terms of stability, with a duration 16 times longer. In SMLM imaging, **MAO-N$_3$** allows the resolution of discrete IMM cristae structures. Additionally, in STED microscopy, **MAO-N$_3$** provides a clearer observation of the individual IMM cristae. These findings suggest that the **HIDE** probe exhibits non-toxic, photostable, and cell-permeable properties, making it a valuable tool for prolonged investigations into IMM dynamics and inter-organelle interactions. The **MAO-N$_3$** high-density environment-sensitive probe presented in this article offers a long-duration, high-resolution tool for mitochondrial inner membrane imaging. However, its complex synthesis pathway, as well as its long-term stability and specificity in complex biological environments, require further assessment.

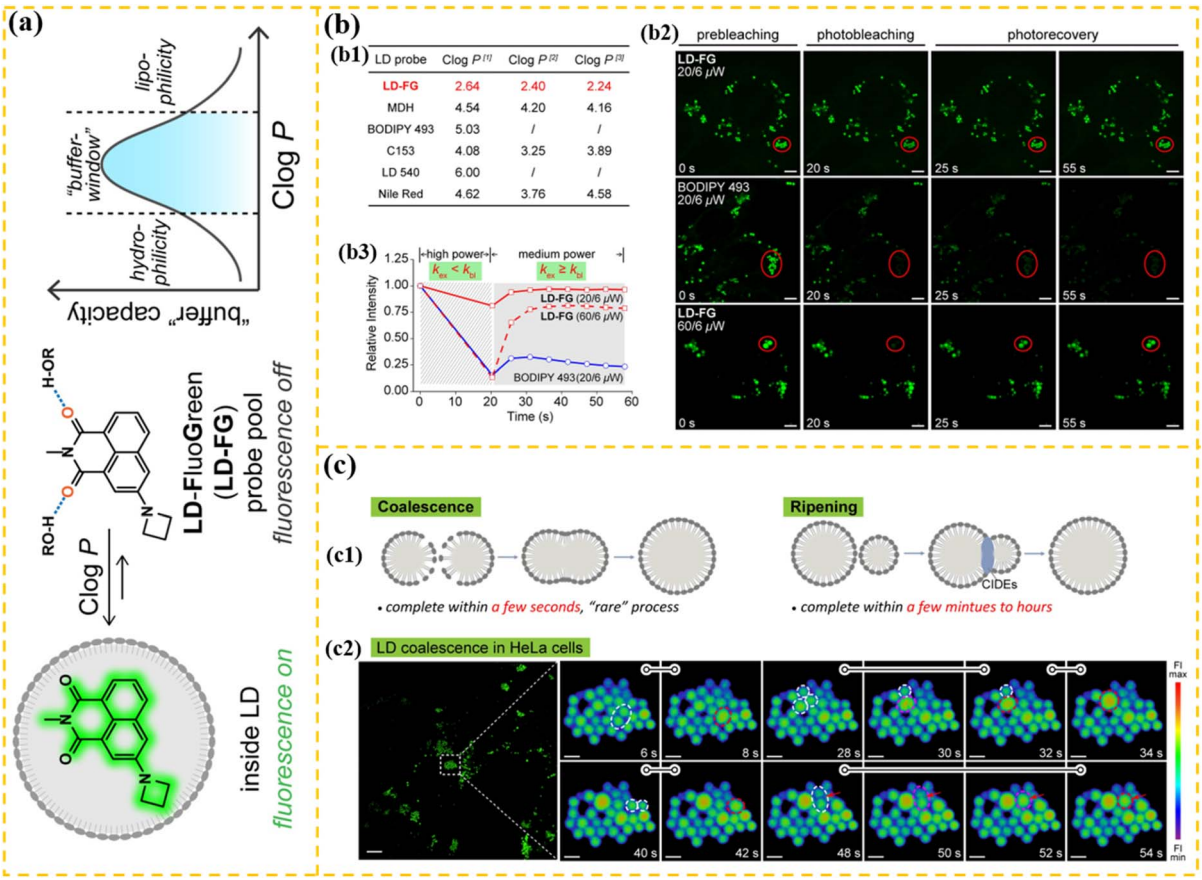

Fig. 29 (a) Chemical structure of probe **LD-FG** and the buffering strategy of the probe. (b) SIM imaging of probe **LD-FG** during photobleaching and photorecovery in HepG2 cells. (b1) The calculated *C* log *P* values for probe **LD-FG** and additional lipid droplet probes using Chemdraw, XLOGP3 and Molinspiration calculations. (b2) SIM imaging of probe **LD-FG** during photobleaching and photorecovery of live HepG2 cells. (b3) Fluorescence intensity ratio of probe **LD-FG** in the red-circled region of (b2) during photobleaching and photorecovery of live HepG2 cells. (c) Imaging of the dynamic aggregation process of probe **LD-FG** in live HeLa cells. Reprinted with permission from Wiley, copyright 2021, as cited in ref. 86.

3.1.2 Fluorescent probes for SIM imaging of lipid droplets. Lipid droplets are crucial cellular structures involved in energy storage and metabolic regulation, playing a key role in lipid metabolism, cellular stress responses, and disease progression. As research advances, their involvement in various physiological and pathological processes, particularly in metabolic diseases and tumors, is being increasingly uncovered.[80] Common fluorescent probes, such as Nile Red and various BODIPY-based probes, have established a solid presence in the field of lipid droplet detection due to their high sensitivity and specificity. These probes can rapidly penetrate cells or tissues, effectively staining lipid droplets, thus allowing researchers to visually observe their presence and distribution. However, despite the excellent performance of these probes in detecting lipid droplets, there are certain limitations in capturing the dynamic alterations of lipid droplets and their interplay with other organelles. In recent years, the emergence of super-resolution lipid droplet probes has brought about a revolutionary breakthrough in lipid droplet research. These probes can capture lipid droplet details at the nanoscale, enabling multi-color imaging and long-term stable imaging, thereby greatly expanding the depth and breadth of lipid droplet studies.[81,82] Consequently, to observe dynamic changes and fine structures of lipid droplets with greater precision, super-resolution imaging technologies have emerged, with Structured Illumination Microscopy (SIM) providing unprecedented spatial resolution for lipid droplet research. When combined with fluorescent probes, SIM imaging not only effectively tracks the formation, dynamic transformation, and interactions of lipid droplets with other cellular structures, but also allows for efficient and real-time observation at the cellular or tissue level (as shown in Fig. 25). Through these advanced techniques, we gain deeper insights into the crucial roles of lipid droplets in cellular health and disease, providing more accurate tools for research and clinical applications in relevant fields.

In 2021, Dong and colleagues designed and produced a donor–acceptor type AIEgen probe, **DTPA-BTN**, based on aggregation-induced emission (AIE) luminogens (AIEgens). This probe exhibits near-infrared emission properties and high photoluminescence quantum yield (PLQY = 11.35%), demonstrating excellent photostability. Building on this AIE aggregate, the authors further encapsulated **DTPA-BTN** into nanoparticles, creating **DTPA-BTN-NPs**.[83] This nanoprobe shows lipid droplet targeting capabilities and excellent biocompatibility, making it highly suitable for super-resolution SIM imaging (as shown in Fig. 26). This work paves the way for further exploration of AIE

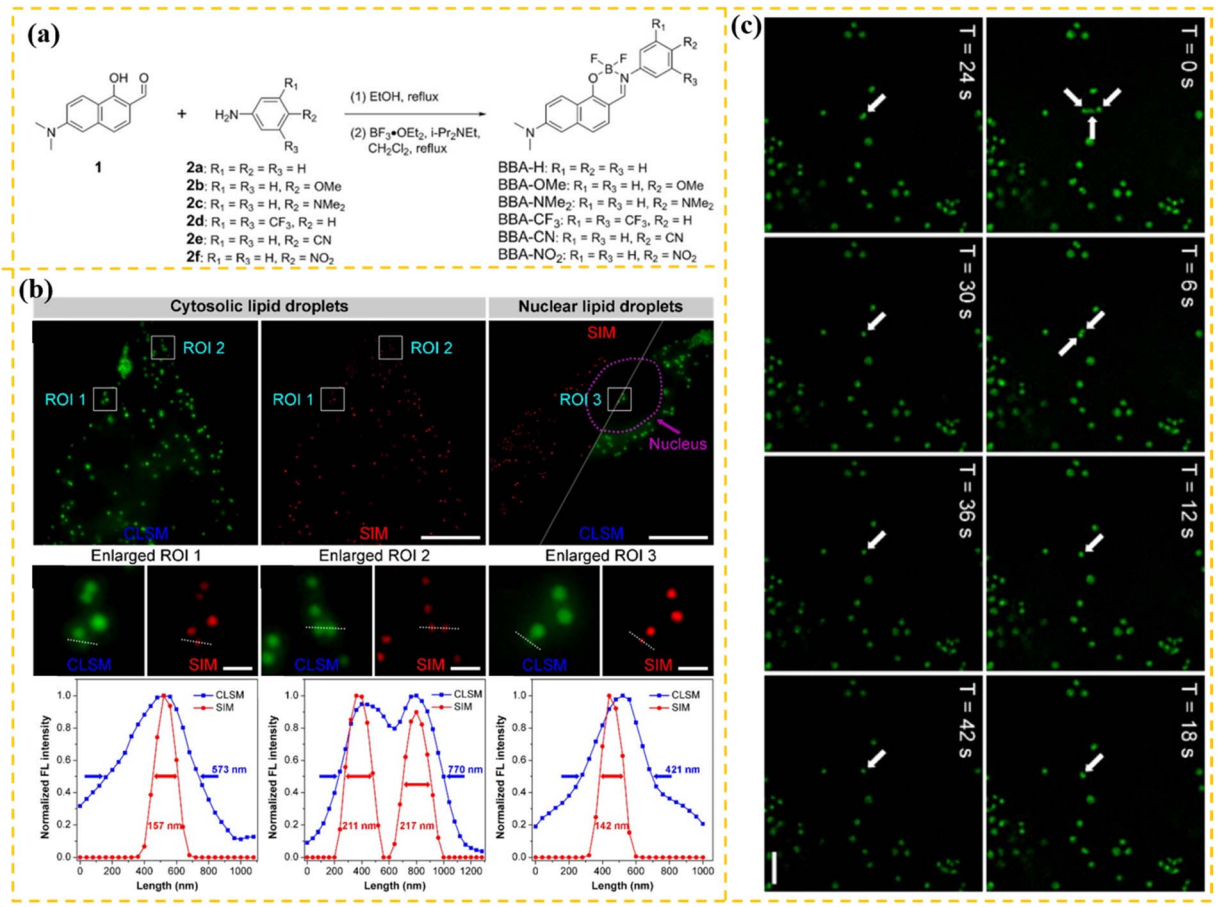

Fig. 30 (a) Synthesis route of probe **BBA**. (b) Super-resolution fluorescence imaging of probe **BBA-CF_3** in HeLa cells following oleic acid stimulation. (c) Dynamic tracking process of probe **BBA-CF_3** in HeLa cells following oleic acid stimulation. Reprinted with permission from the American Chemical Society, copyright 2022, as cited in ref. 87.

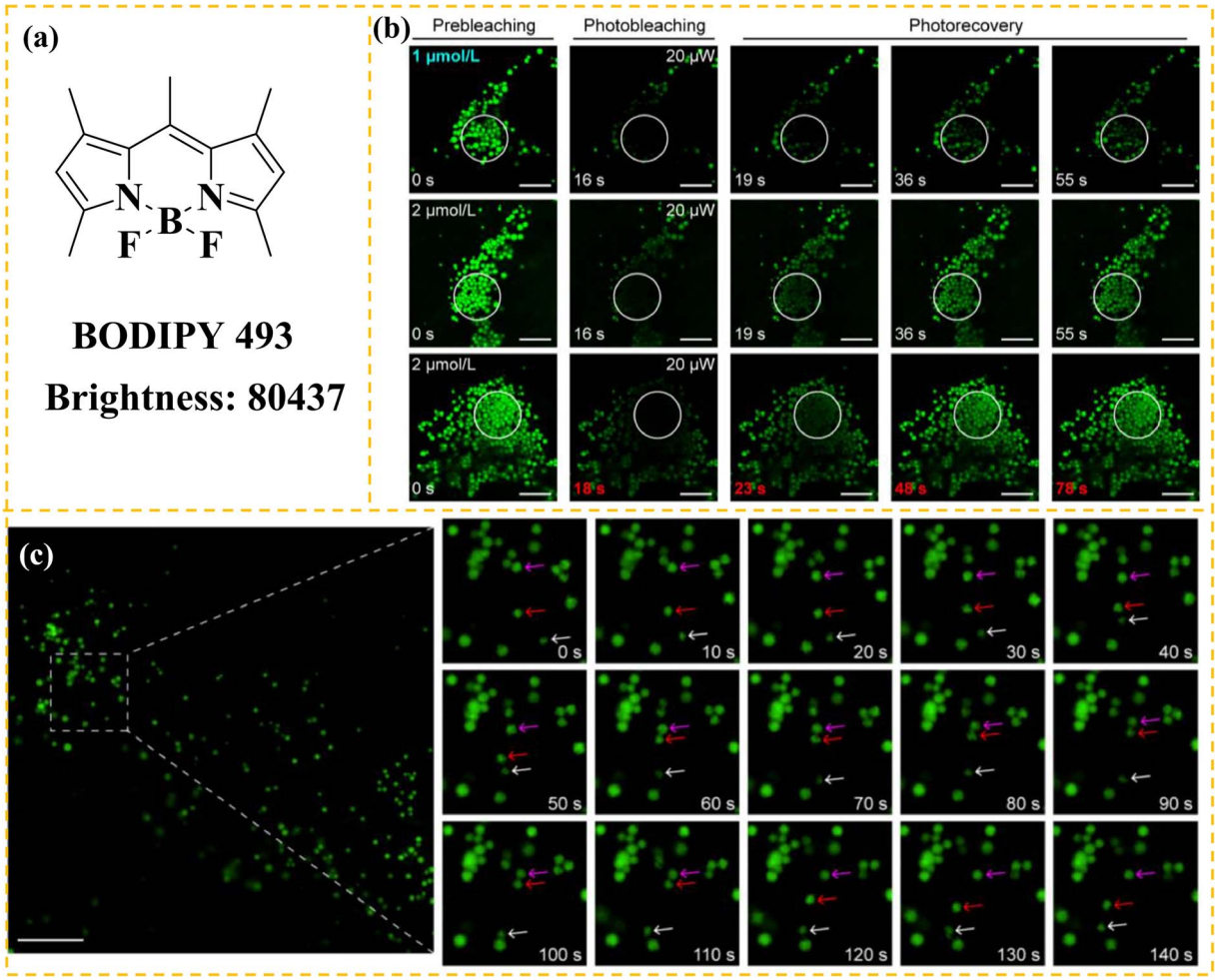

Fig. 31 (a) Chemical structure and fluorescence brightness parameters of compound **BODIPY 493**. (b) SIM imaging of **BODIPY 493** in live HeLa cells during photobleaching and subsequent photorecovery. (c) Dynamic time-lapse SIM imaging of **BODIPY 493** in live HeLa cells. Reprinted with permission from Elsevier, copyright 2022, as cited in ref. 88.

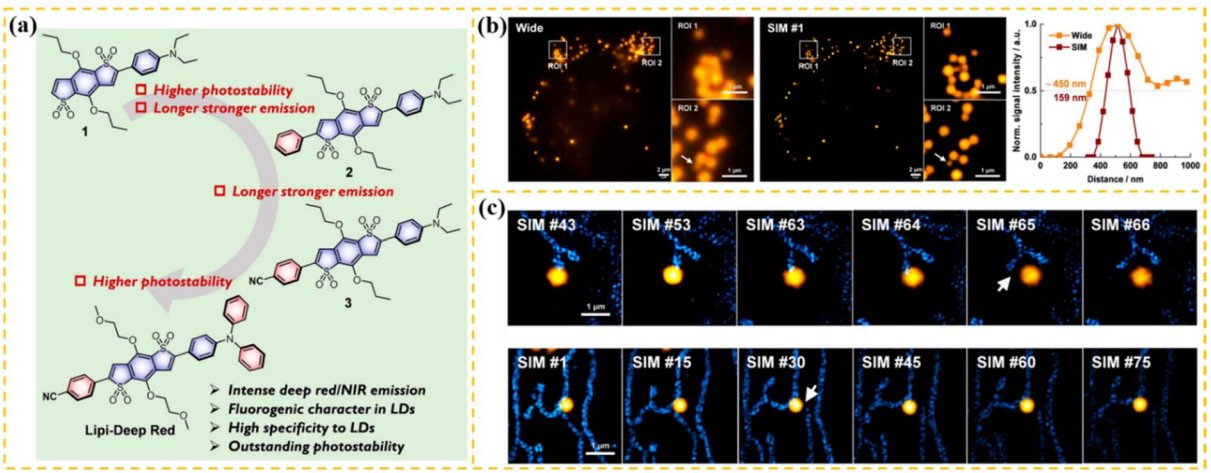

Fig. 32 (a) Design strategy of probe **Lipi-Deep Red**. (b) Super-resolution fluorescence imaging of probe **Lipi-Deep Red** in HeLa cells. (c) Dual-color SIM imaging of probe **Lipi-Deep Red** with mitochondrial commercial dye MTG in HeLa cells and dynamic time-lapse imaging of lipid droplet–mitochondria interaction. Reprinted with permission from Elsevier, copyright 2023, as cited in ref. 89.

aggregates in biological research applications. In the future, with the integration of multimodal imaging and smart responsiveness, AIEgen-based probes are expected to play a crucial role in the diagnosis of metabolic diseases, cell signal transduction analysis, and clinical surgical navigation, advancing biomedical imaging technologies toward greater precision and intelligence.

In the same year, Chen and co-workers developed a near-infrared fluorescent probe **DTZ-TPA-DCN**, based on the D–π–A framework for SIM imaging of lipid droplets.[84] As shown in Fig. 27, the probe was mainly designed by the conjugated linkage of tritylamine as an electron donor, cyano as an electron acceptor, and a thiophene and C=C double bond. The probe **DTZ-TPA-DCN** has excellent photostability and can be used for SIM imaging of lipid droplets. In addition, the probe can be successfully applied not only to study the kinetic imaging under ER stress or INM lipid metabolism, but also to image the formation process of lipid droplets under DAG induction. This research offers a dependable tool with great significance for exploring the applications of lipid droplets. However, its capability to investigate the dynamic changes in nuclear lipid droplets and its specificity in complex biological systems still require further validation and optimization.

Next, Chen and colleagues developed a fluorescent probe **C-Py**, based on coumarin as a fluorescent moiety for SIM imaging of lipid droplets.[85] Due to the excellent optical properties of the probe, it can be successfully used to monitor the dynamic process of lipid droplets in living cells. The contact processes between the probe **C-Py** and other organelles labeled with commercial dyes were successfully revealed by SIM imaging. In particular, the contact and separation process between lipid droplets and mitochondria in cells stimulated by LPS was observed (shown in Fig. 28). This work holds considerable importance for forthcoming investigations into the diagnosis and therapeutic interventions of lipid droplet-related diseases. However, its ability to comprehensively analyze lipid droplet dynamics and interactions with other organelles, as well as its efficacy in complex disease models, still requires further in-depth investigation and validation.

Xu *et al.* designed and synthesized a fluorescent probe, **LD-FG**, based on naphthalimide as the fluorescent parent for SIM imaging of lipid droplet dynamics.[86] The probe **LD-FG** has a hydrogen bond-sensitive fluorescence response, which enables wash-free imaging of lipid droplets. The probe is appropriately lipophilic and has a $C\log P$ value within the "buffer window" of 0–3, which can be used to compensate for the photobleaching process for long-term stable SIM imaging. In addition, the probe was used to successfully monitor the dynamic interaction process of lipid droplets. For the first time, the "dumbbell-shaped" intermediate pattern of the lipid droplet aggregation process was initially visualized within cells, and it was found that this process is not a rare dynamic process, but one that occurs frequently in cells (Fig. 29). This study establishes a crucial foundation for investigating the role of lipid droplets in living cells. Overall, the hydrogen-bond-sensitive fluorescent probe **LD-FG** proposed in this article enables high-stability super-resolution imaging of lipid droplet dynamics. However, its ability to explain the specific molecular mechanisms during lipid droplet dynamics, as well as its

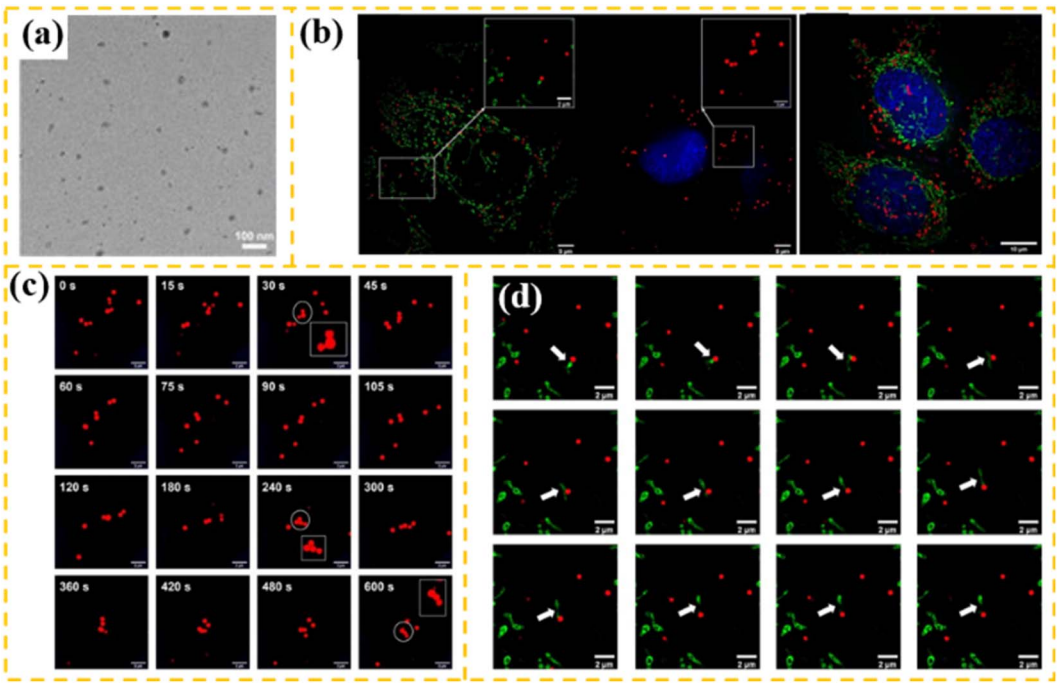

Fig. 33 (a) TEM characterization of the nano-probe **CPD**. (b) Specific SIM imaging of the probe **CPD** in HepG2 cells. (c) Monitoring the dynamic process of lipid droplets in HepG2 cells using the probe **CPD**. (d) SIM imaging of the dynamic interaction process between the probe **CPD** and the commercial mitochondrial dye MTG in HepG2 cells. Reproduced with permission from American Chemical Society, copyright 2024, as cited in ref. 90.

applicability across different cell types and pathological conditions, still requires further exploration and validation.

In 2022, Yu *et al.* designed and synthesized a series of fluorescent probe **BBA**, tailored for specific SIM imaging of lipid droplets based on the D–A architecture.[87] The series of probes was designed and synthesized from an azoxyfluoroboron dye with a rigid structure as a fluorescent moiety by introducing different aniline groups as a donor and exhibited ultra-high fluorescence quantum yields. One of the probes **BBA-CF$_3$** was able to be used for super-resolution fluorescence imaging of lipid droplets and was effectively employed in the dynamic analysis of these droplets (shown in Fig. 30). This work provides an important basis for the precise study of biologically relevant events in lipid droplets, which has great potential. Overall, the **BBA** series of highly luminescent solvent-sensitive fluorescent probes developed in this article demonstrate excellent performance in lipid droplet imaging. However, their synthesis complexity, potential issues with photobleaching, and stability across different cellular environments still require further optimization and comprehensive evaluation.

In December of the same year, Xu *et al.* used the commercial dye **BODIPY 493** as a fluorescent probe to study its dynamic behavior within cells.[88] As shown in Fig. 31, **BODIPY 493** effectively targets lipid droplets, exhibits high brightness, and requires very low laser power for imaging. Additionally, the study revealed that **BODIPY 493** possesses buffering capabilities, allowing for its stable and long-term use in lipid droplet SIM imaging. This article presents the application of **BODIPY493** as a bright buffering fluorescent probe for dynamic super-resolution imaging of lipid droplets. However, its mechanism for probing lipid droplet dynamics remains insufficiently explored, and further research is needed to improve its photostability during long-term imaging and to assess its potential cytotoxicity.

In 2023, Lu *et al.* developed a novel lipid droplet fluorescent probe **Lipi-Deep Red** grounded in a D–π–A–π–A skeleton for super-resolution fluorescence imaging of lipid droplets.[89] As shown in Fig. 32, the probe has excellent photochemical properties and was able to be used for SIM imaging of lipid droplets with a high resolution of 159 nm. In addition, the probe was successfully used to monitor the dynamic processes of lipid droplets. Finally, two-color SIM imaging was successfully achieved using the probe **Lipi-Deep Red** and a mitochondrial commercial dye and revealed the dynamic mutual contact process between lipid droplets and mitochondria. Overall, this study not only highlights the significant potential of **Lipi-Deep Red** as a fluorescent probe but also offers new insights into the design and application of fluorescent probes. However, the **Lipi-Deep Red** applicability across different cell types still requires further validation and optimization.

In 2024, Xu *et al.* developed a fluorescent nanoprobe (**CPD**) of carbonized polymer dots for super-resolution fluorescence imaging of lipid droplets.[90] The probe **CPD** has good biocompatibility and high sensitivity to polarity, and can specifically target lipid droplets. In addition, the authors successfully observed the dynamic changes in lipid droplets in living cells using the probe. At the same time, the mutual contact process between mitochondria and lipid droplets was successfully monitored (as shown in Fig. 33). This work helps us further understand the biological functions of lipid droplets and offers new insights for developing novel super-resolution fluorescent probes. Overall, this article demonstrates the use of **CPD** for super-resolution imaging of lipid droplets in live cells, highlighting their potential for tracking lipid droplet dynamics and interactions with other organelles. However, their ability to resolve specific molecular events during lipid droplet dynamics and their applicability under various physiological and pathological conditions still require further investigation.

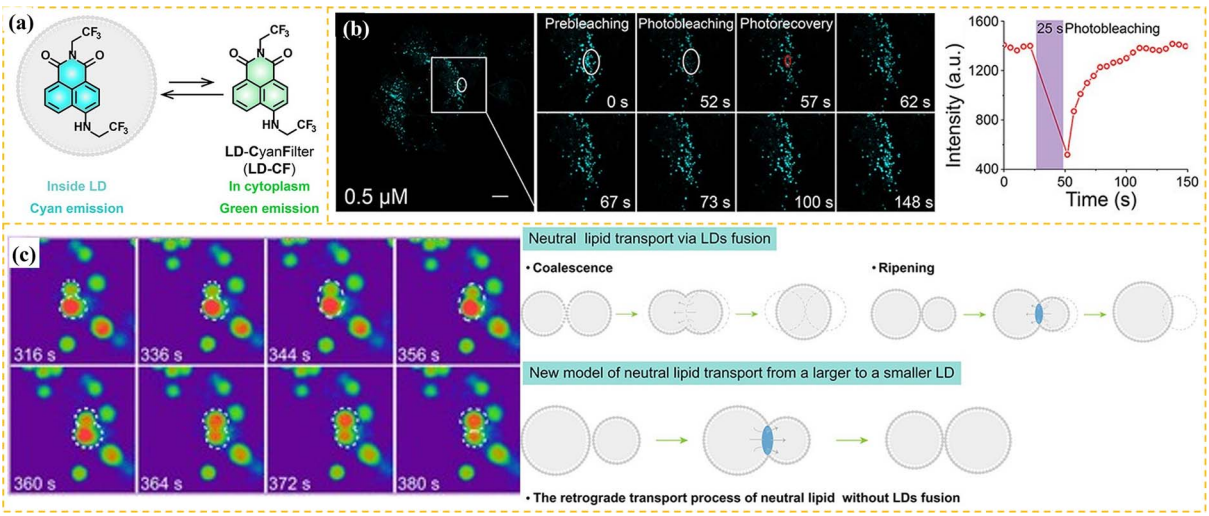

Fig. 34 (a) Mechanism of the LD-CF probe response to lipid droplets and probe response to polarity. (b) Buffer imaging of probe LD-CF in HeLa cells. (c) Probe LD-CF is used for monitoring the dynamic process of lipid transport between lipid droplets in HeLa cells through SIM imaging. Reprinted with permission from American Chemical Society, copyright 2024, as cited in ref. 91.

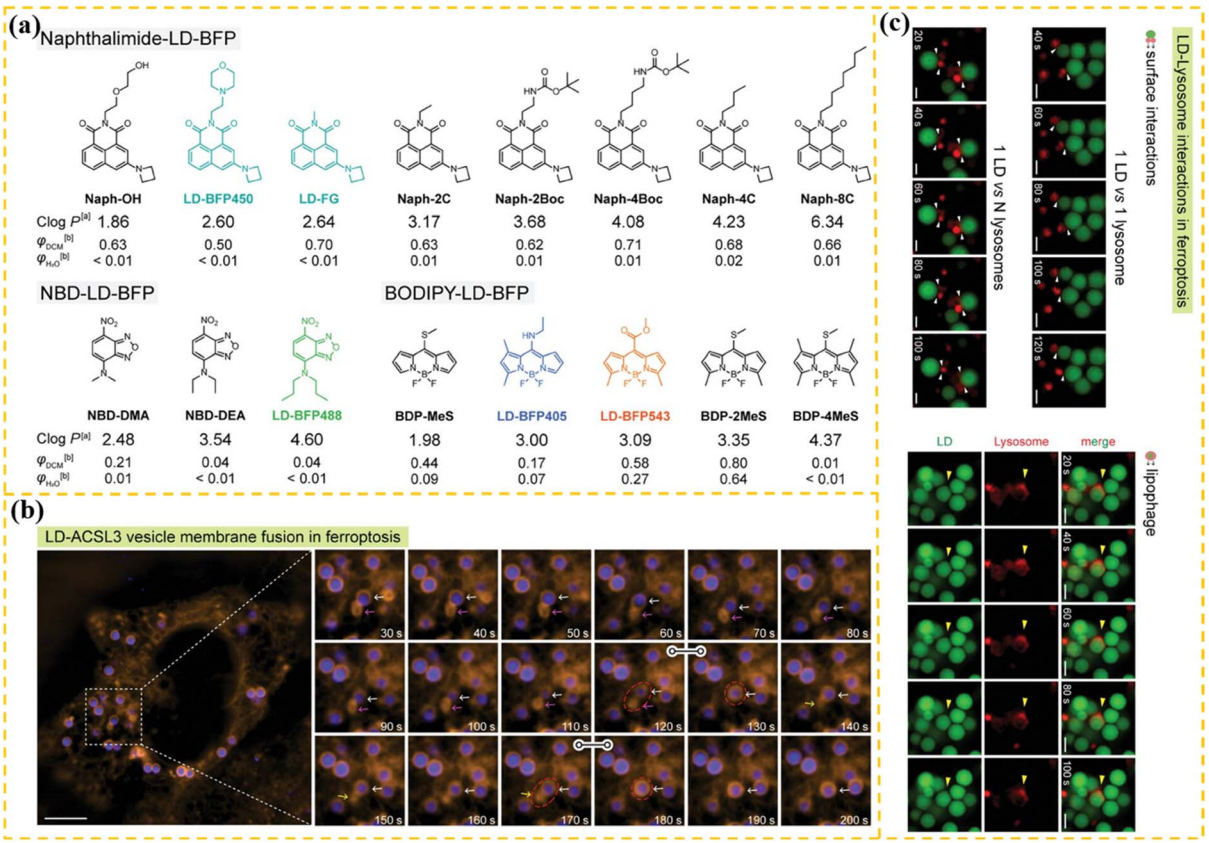

Fig. 35 (a) Chemical structures and optical parameters of various lipid droplet probes LD BFPs. (b) SIM imaging of the interaction between probe **LD-BFP405** and ACSL3 protein in cell iron death. (c) Imaging of the interaction between lipid droplets and lysosomes during cell iron death using probe **LD-BFP488** and lysosomal commercial dye. Reprinted with permission from Wiley, copyright 2024, as cited in ref. 92.

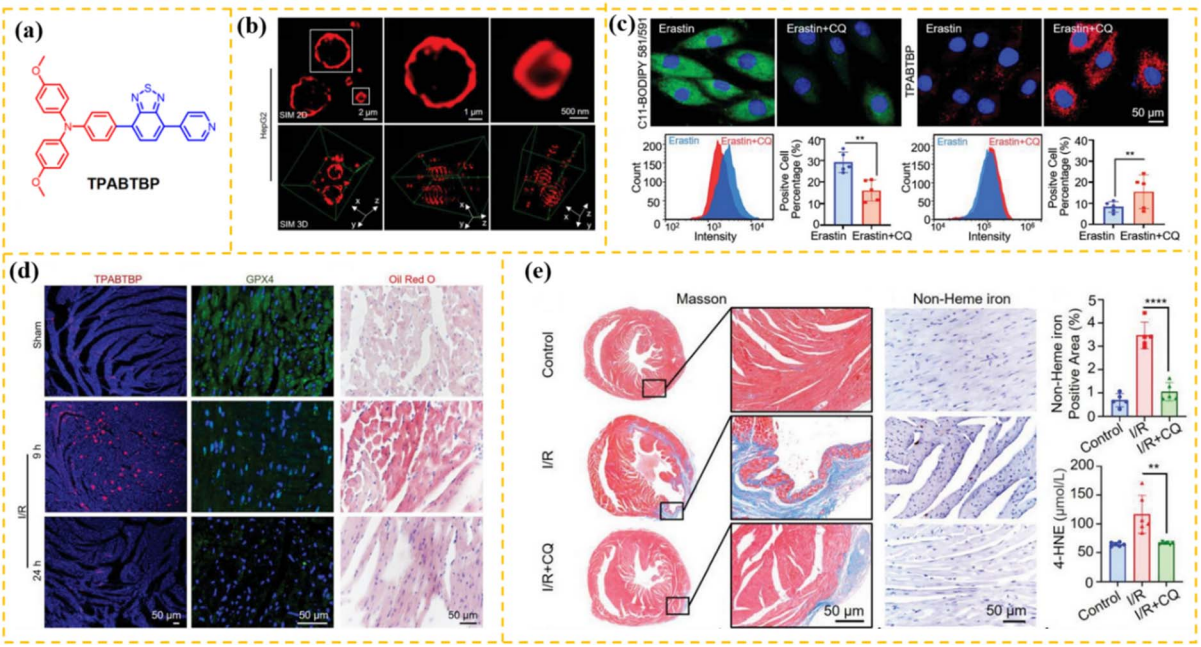

Fig. 36 (a) Chemical structure of probe **TPABTBP**. (b) 2D and 3D SIM imaging of probe **TPABTBP** in HepG cells. (c) Lipid droplet imaging of the **TPABTBP** probe during cell iron death and imaging of chloroquine inhibition of the lipid droplet degradation process. (d) Imaging of probe **TPABTBP** in I/R damaged hearts incubated at different times. (e) Fluorescence imaging of MIRI mouse hearts after CQ treatment. Reprinted with permission from Wiley, copyright 2024, as cited in ref. 93.

During that same year, Xu and colleagues introduced a buffer probe **LD-CF** for SIM imaging of lipid droplet dynamics.[91] The probe is designed and synthesized using naphthalimide substituted with trifluoromethylamine, which is sensitive to polarity and its wavelength increases with increasing polarity. Using the probe **LD-CF** as a buffer pool, wash-free and long-term dynamic super-resolution imaging of lipid droplets was accomplished, and the fusion process of large lipid droplets flowing towards small lipid droplets was observed (as shown in Fig. 34). In addition, the lipid transport process between lipid droplet pairs in live cells was successfully revealed by the probe **LD-CF**. This study provides strong support for deciphering and understanding the dynamic physiological processes of organelles in the future. Overall, this study not only demonstrates the potential application of the **LD-CF** probe in lipid droplet research but also provides new technical insights and tools for the in-depth investigation of inter-organelle interactions and associated physiological processes. However, its detailed molecular mechanism for lipid droplet dynamics remains insufficiently explained, and its stability and specificity across different cellular environments require further experimental validation.

Subsequently, the team further explored the key factors affecting the buffering capacity of LD probes and developed a multi-color fluorescent probe for LD.[92] As shown in Fig. 35, by evaluating the lipophilicity parameter ($C\log P$ value) of fluorescent dyes, it was found that dyes with $C\log P$ values between 2.5 and 4 have the best staining buffering ability. The study screened multiple fluorescent dyes and discovered four different colored LD-buffered fluorescent probes, which have moderate lipids and high photostability and are suitable for super-resolution fluorescence imaging. Through SIM imaging, it was found that the ACSL3 protein may interact with lipid droplets in the form of vesicles during ferroptosis and participate in maintaining cellular homeostasis. These probes provide new tools for visualizing LD dynamics and contribute to a deeper understanding of LD biology in cellular processes and related diseases. Although this article successfully develops a multicolor buffering fluorescent probe for lipid droplet dynamic imaging using the $C\log P$ parameter, it lacks an in-depth discussion on the probe's nonspecific binding in complex biological systems, potential toxicity, and photostability during long-term imaging, which limits its broader applicability.

In addition, Xie *et al.* developed a lipid droplet fluorescent probe **TPABTBP** based on the combination of intramolecular charge transfer (ICT) and AIE, which is used to track the dynamic alterations in lipid droplets occurring during ischemia/reperfusion-induced myocardial cell ferroptosis.[93] This probe has excellent lipid droplet targeting and photostability. Through super-resolution SIM imaging, the probe **TPABTBP** can specifically stain phospholipid monolayers. In the MIRI mouse model, it can be observed that the level of lipid droplets increases in the early stage of reperfusion, while it decreases in the late stage through lipid phagocytosis. In addition, the use of chloroquine to inhibit the iron death

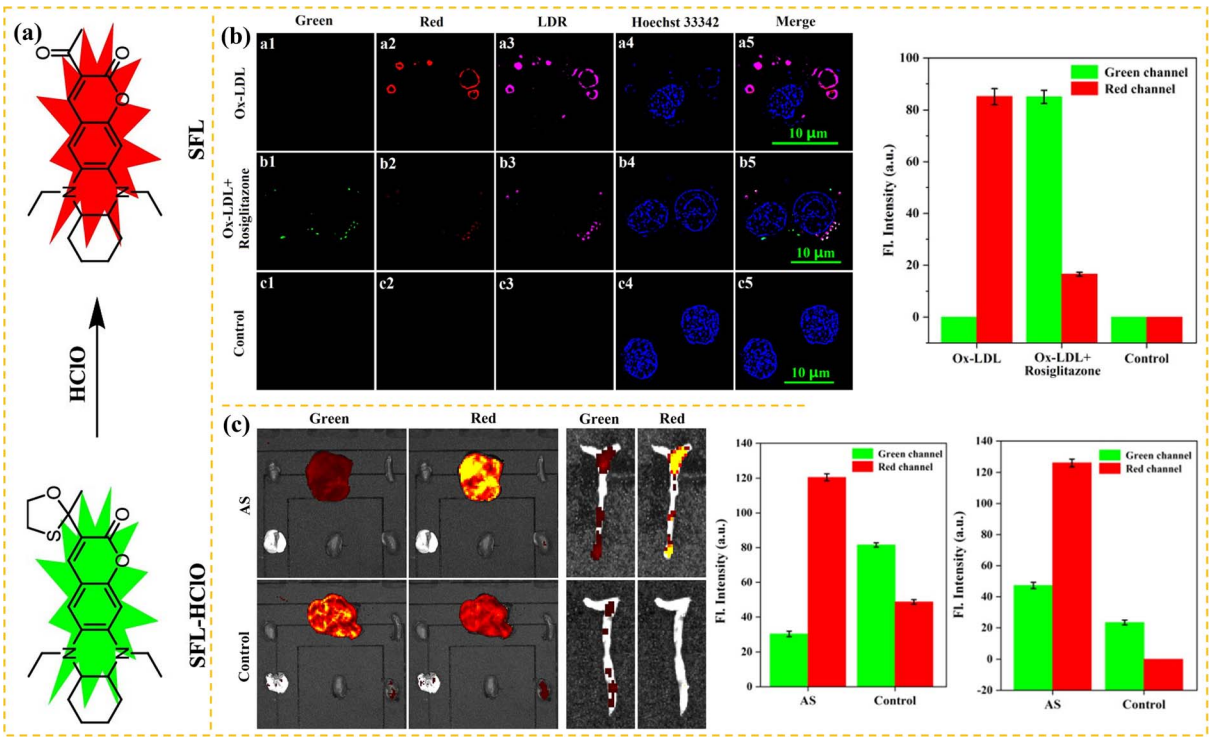

Fig. 37 (a) Chemical structure of the **SFL-HClO** probe and its reaction mechanism with HClO. (b) The probe **SFL-HClO** was employed to visualize HClO within foam cells using SIM imaging techniques. (c) Probe **SFL-HClO** was used to image HClO in atherosclerotic mouse models. Reprinted with permission from American Chemical Society, copyright 2024, as cited in ref. 94.

process resulted in a decrease in MIRI-induced iron death in mice (as shown in Fig. 36). This work provides useful assistance for potential therapeutic targets of early MIRI intervention. This article reveals the dynamic changes in lipid droplets in myocardial ischemia/reperfusion injury through the development of the **TPABTBP** probe. However, the in-depth mechanistic analysis of the complex interactions between lipid droplets and the autophagy pathway is insufficient, and the long-term safety and efficacy of the probe in *in vivo* applications still require further validation.

Atherosclerosis (AS) is the key factor that causes cardiovascular disease. One of its typical pathological characteristics is atherosclerotic plaque, which is mainly composed of foam cells, and these cells are characterized by abnormal increases in lipid droplets (LD) and hypochlorite (HClO) levels. Hence, it is of great significance to employ super-resolution imaging to study the changes in lipid droplets and HClO during atherosclerosis. Building upon this, Yang and colleagues devised and developed a ratio-type fluorescent probe **SFL-HClO** for SIM imaging of lipid droplets and HClO.[94] As shown in Fig. 37, the probe successfully revealed the fine structure of cellular LD through super-resolution imaging technology. In addition, the probe **SFL-HClO** can respond to LD and HClO in a specific ratio, which is helpful for accurate imaging of foam cells. Importantly, the probe can also be used to monitor the changes in HClO in the process of atherosclerosis, which holds considerable importance for the early diagnosis of atherosclerosis. This study offers a novel approach for the early diagnosis of atherosclerosis by monitoring changes in HClO levels, which may facilitate the early detection of atherosclerotic progression, thereby providing substantial support for clinical treatment. However, the specific mechanistic role of HClO in atherosclerosis progression and the probe's ability to selectively recognize targets in complex biological environments still require further in-depth investigation.

During that same year, Chen and colleagues adopted a three-pronged innovative strategy and successfully constructed an **LDM** pre-probe.[95] This probe can accurately locate and deliver to the LD membrane area based on its specific physicochemical properties. Experimental data show that in the LD microenvironment, the **LDM** precursor probe can be activated by HClO and transformed into a functional **LDM-OH** probe. Further research has found that **LDM-OH** specifically binds to LD membrane-related proteins through electrostatic attraction,

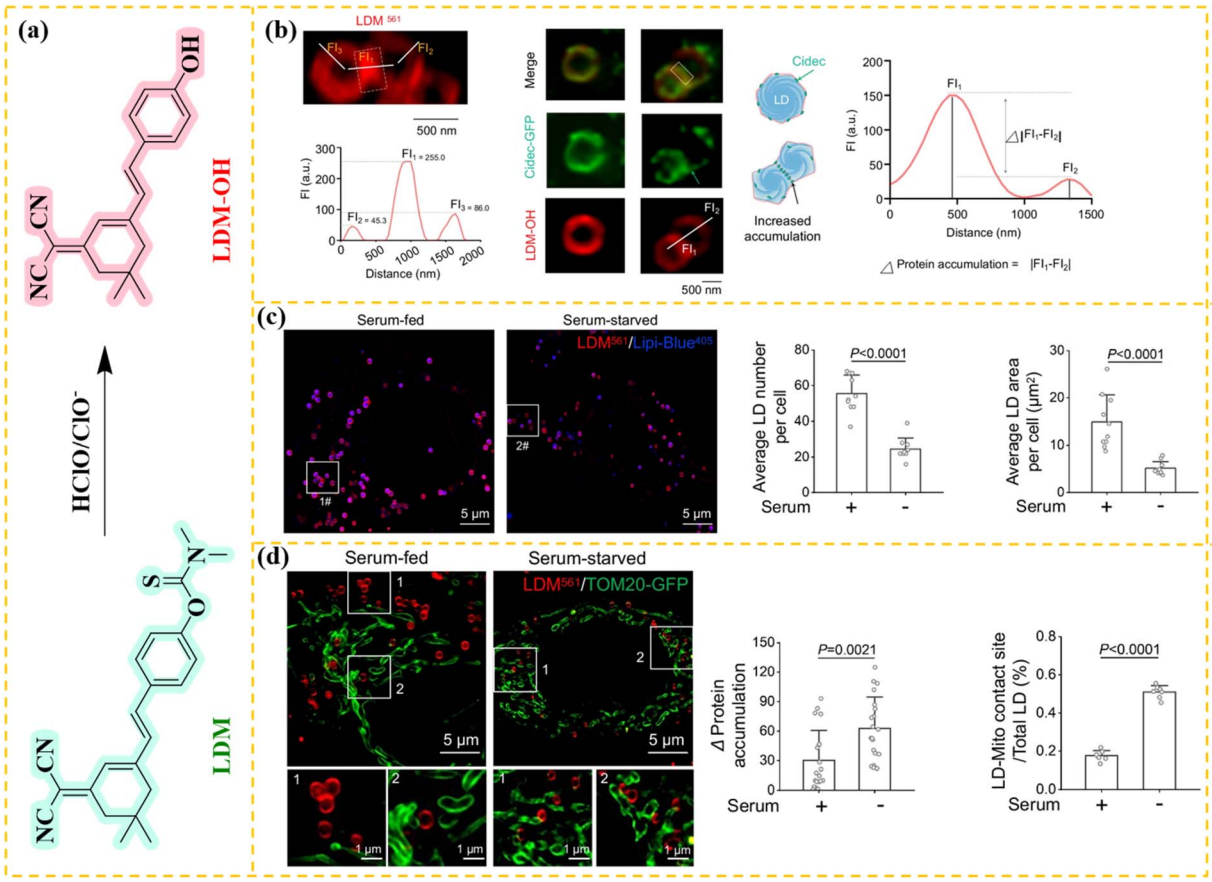

Fig. 38 (a) Chemical structures of probe **LDM** and precursor **LDM-OH**, as well as the response mechanism between **LDM** and HClO. (b) SIM imaging using the **LDM** probe as a parameter indicator for membrane contact protein accumulation in living cells. (c) SIM imaging and quantitative analysis of probe **LDM** and Lipi-B405 in HepG2 cells induced by hunger. (d) SIM imaging and quantitative analysis of lipid droplets and mitochondria labeled with **LDM** and TOM20-GFP in HepG2 cells induced by hunger. Reprinted with permission from Springer Nature, copyright 2024, as cited in ref. 95.

achieving highly selective visualization of the LD membrane. With the help of this probe system, the team successfully revealed the protein aggregation phenomenon of the LD membrane in living cells, and verified the dynamic contact mechanism between the LD membrane and mitochondria and their related protein aggregation parameters (Fig. 38). In addition, the correlation between protein aggregation characteristics in the membrane contact area and membrane protein content per unit area was also explored in depth. Overall, although the **LDM** probe proposed in this article can specifically label lipid droplet membranes and reveal their dynamic mechanisms, its applicability across different cell types and physiological/pathological conditions, long-term imaging stability, and capability for in-depth analysis of lipid droplet membrane-related molecular events still require further validation and optimization.

3.1.3 Fluorescent probes for lysosome SIM imaging. Lysosomes, as important organelles within cells, participate in key physiological processes such as substance degradation, endocytosis, and autophagy.[96,97] With the deepening of cell biology research, the function and dynamic behavior of lysosomes has attracted increasing attention. However, due to the small size of lysosomes and their complex and interwoven morphology with other organelles, traditional fluorescence imaging techniques face significant challenges in high-resolution observation. In recent years, advancements in super-resolution imaging methodologies, including structured light illumination microscopy (SIM), have enabled researchers to surpass the optical diffraction limit and obtain fine structure and dynamic information of lysosomes. To more accurately observe the morphology and function of lysosomes, the design and application of fluorescent probes have become particularly important (as shown in Fig. 39). Fluorescent probes specifically used for lysosome imaging should not only have good lysosome targeting properties, but also excellent optical properties including high fluorescence intensity, good photostability, and suitable fluorescence emission wavelengths.[98–100] Through these fluorescent probes, combined with super-resolution imaging technology, researchers can monitor the dynamic changes in lysosomes in real-time and analyze their roles in different physiological and pathological states, providing new perspectives and powerful tools for understanding various biological processes within cells.

In 2022, Diao *et al.* designed and synthesized nanoclusters **BSA NCs** for SIM imaging of lysosomes by modifying metal Au and Ag with bovine serum albumin.[101] This probe has high photostability and can be used for long-term dynamic studies of mitochondria and lysosomes in autophagy processes. The probe **BSA-NCs** has pH anti-interference ability, and regardless of pH fluctuations in lysosomes, **BSA-NCs** can be stably retained in lysosomes (Fig. 40). In addition, **BSA-Au NCs** can also be used for dynamic imaging of lysosomes in brain organoids. This work provides a method for synthesizing fluorescent probes for ultra-long-time imaging. Overall, this article demonstrates the potential of **BSA-NCs** for tracking lysosome dynamics in super-resolution imaging. However, the specific mechanisms underlying the interactions between lysosomes and other organelles are insufficiently explored, and the applicability and long-term

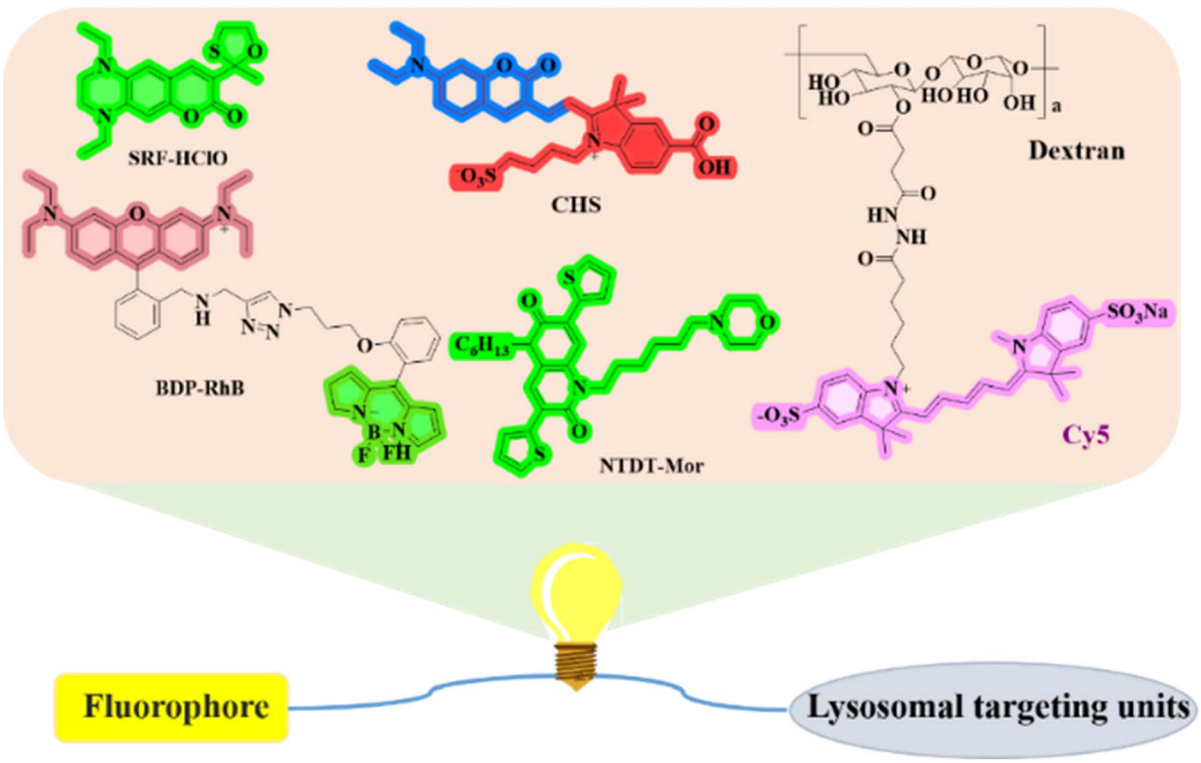

Fig. 39 Design of fluorescent probes for lysosome super-resolution fluorescence imaging and the chemical structure of lysosome probes.

imaging performance in different biological models require further validation.

In 2023, Chen and colleagues created a novel switchable fluorescent probe **BDP-RhB** based on FRET for dynamic SIM imaging of lysosomes.[102] This probe can convert biological signals into color signals through the FRET mechanism and can use SIM imaging technology to monitor the flow of H^+ in the microenvironment during the dynamic interaction between lysosomes and mitochondria in real time (Fig. 41). In addition, the probe BDP RhB can also be used to screen potential MLC regulatory drugs. This work provides a novel approach for identifying drugs that impact lysosomal acidification and mitochondrial calcium (MLC) dynamics. Overall, the **BDP-RhB** probe proposed in this article can monitor the dynamic changes in H^+ at mitochondria–lysosome contact sites in real-time. However, its in-depth analysis of the H^+ transport regulation mechanisms is limited, and the probe's applicability and imaging stability under different cellular conditions still require further exploration.

In 2024, Wang *et al.* designed and synthesized a gold nanocluster using 6-aza-2 thiothymine (ATT) and L-arginine (Arg) as reducing and protecting ligands to form **Arg@ATT-AuNCs** used for SIM imaging of intracellular lysosomes.[103] The author observed the single-particle and aggregation state of **AuNCs** under re-fixed cells using STEM imaging and successfully captured the real-time dynamic aggregation process of **AuNCs** in lysosomes in live cells using 3D-SIM imaging (Fig. 42). Furthermore, imaging revealed that the formation of different aggregation states of **Arg@ATT-AuNCs** was closely related to different pH values in the solution. This investigation presents a novel methodology for exploring the interplay between cells and various nanoparticles. Overall, this article employs STEM and 3D-SIM imaging techniques to reveal the aggregation behavior and toxicity mechanisms of gold nanoclusters within cells. However, the exploration of the specific molecular mechanisms of gold nanocluster aggregation, their differential effects across various cell types, and potential intervention strategies remains insufficient.

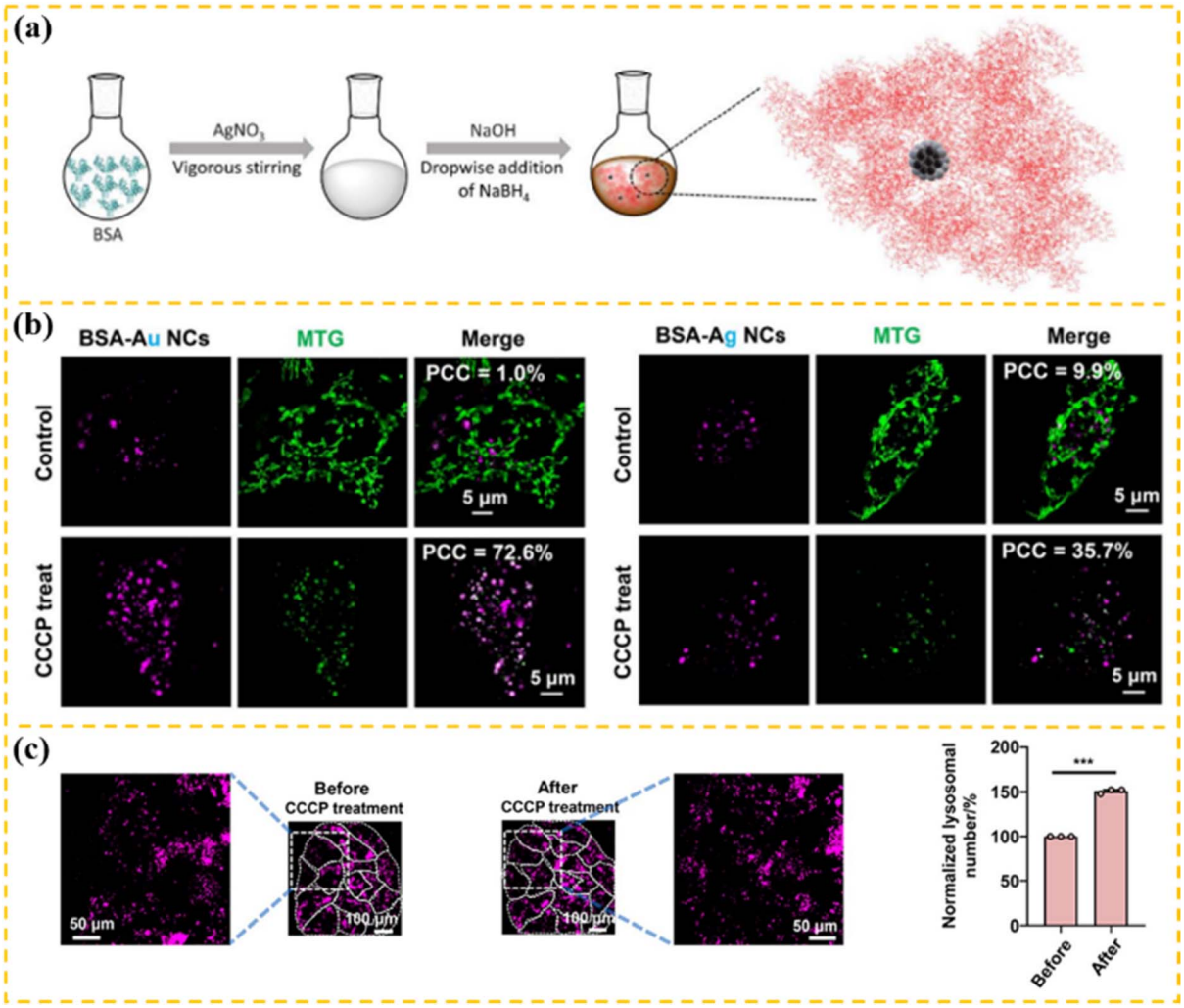

Fig. 40 (a) Synthesis process of **BSA-Ag NCs** nanoclusters. (b) Imaging of the interaction between lysosomes and mitochondria during cellular mitochondrial autophagy using probes **BSA-NCs** and MTG. (c) Dynamic imaging of lysosomes in brain organoids using the **BSA-Au NCs** probe. Reprinted with permission from American Chemical Society, copyright 2022, as cited in ref. 101.

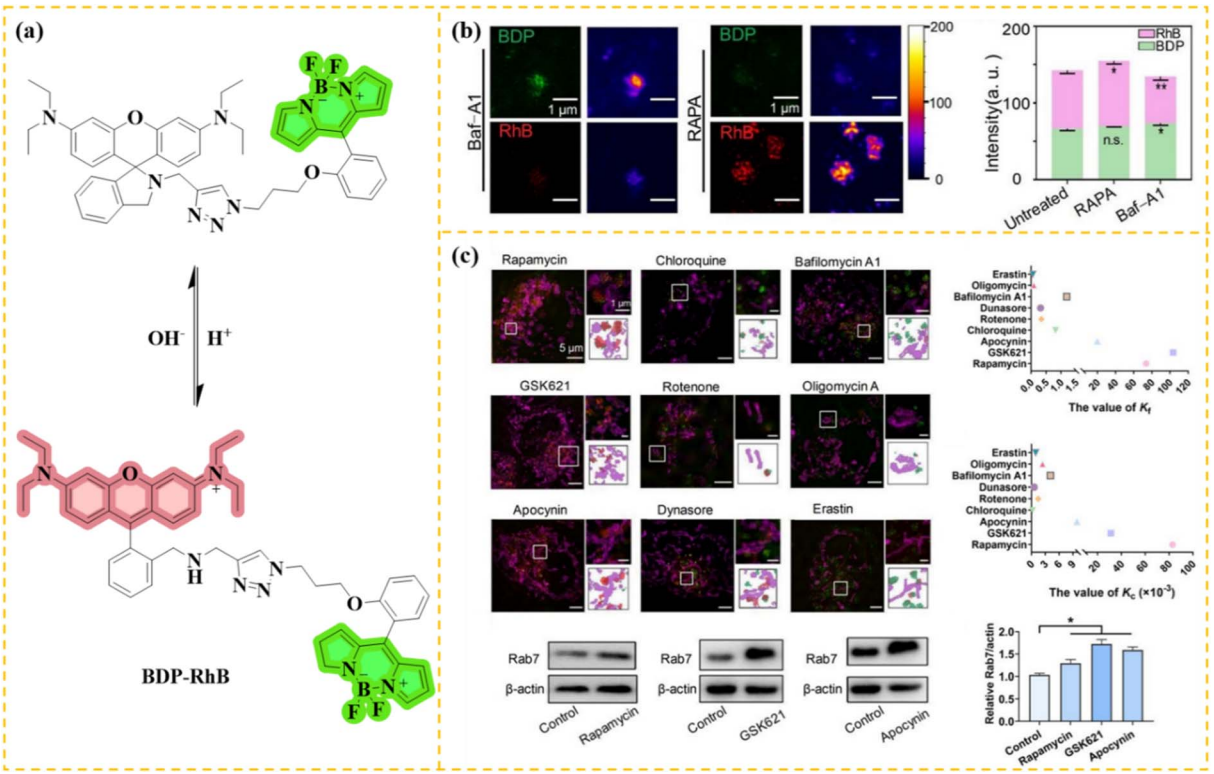

Fig. 41 (a) Chemical structure of probe **BDP-RhB** based on the FRET mechanism. (b) SIM imaging of the **BDP-RhB** probe for regulating the lysosomal pH process by bafilomycin A1 (Baf-A1) and rapamycin (RAPA). (c) Imaging of the drug screening process using probe **BDP-RhB** during mitochondrial lysosome contact (MLC). Reprinted with permission from Wiley, copyright 2023, as cited in ref. 102.

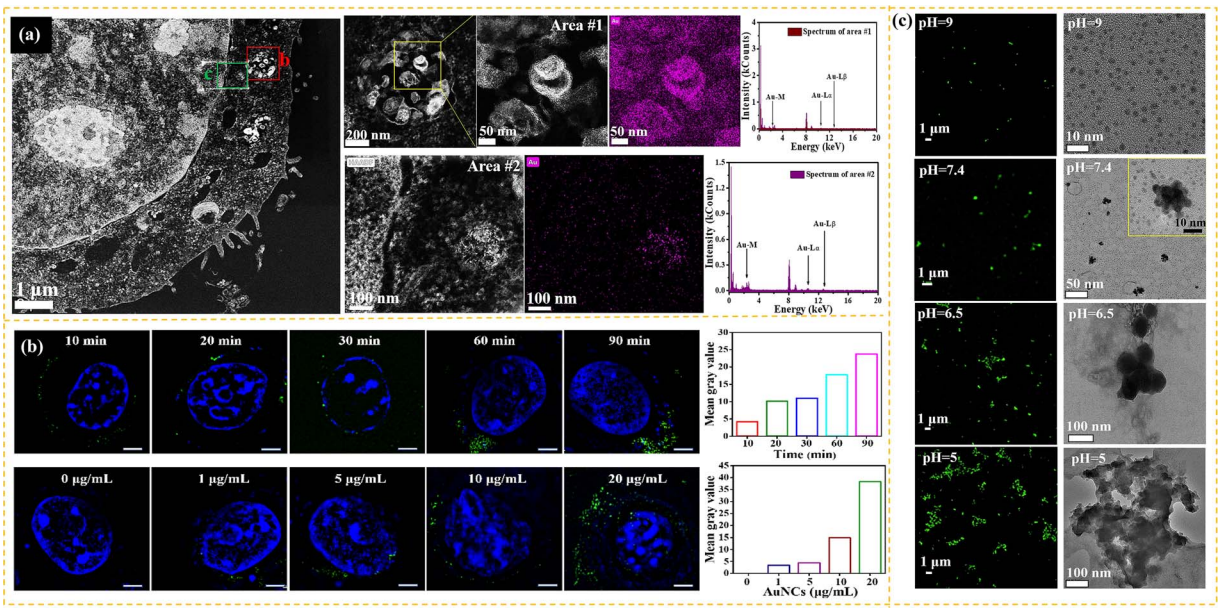

Fig. 42 (a) Gold nanocluster **Arg@ATT-AuNC** STEM imaging within cells. (b) Gold nanocluster **ATT@Arg-AuNCs** SIM imaging of cells under different culture conditions and at different concentrations. (c) Gold nanocluster **Arg@ATT-AuNC** SIM and TEM imaging in solutions with different pH values. Reprinted with permission from Elsevier, copyright 2024, as cited in ref. 103.

During that same year, Chen and colleagues created a fluorescent probe **(FITC)-MOS-1** that combines fluorescein isothiocyanate with the natural product mussel oligosaccharides to overcome the potential biological applications of exploring natural products (NPs), for super-resolution fluorescence imaging of intracellular lysosomes.[104] As shown in Fig. 43, the

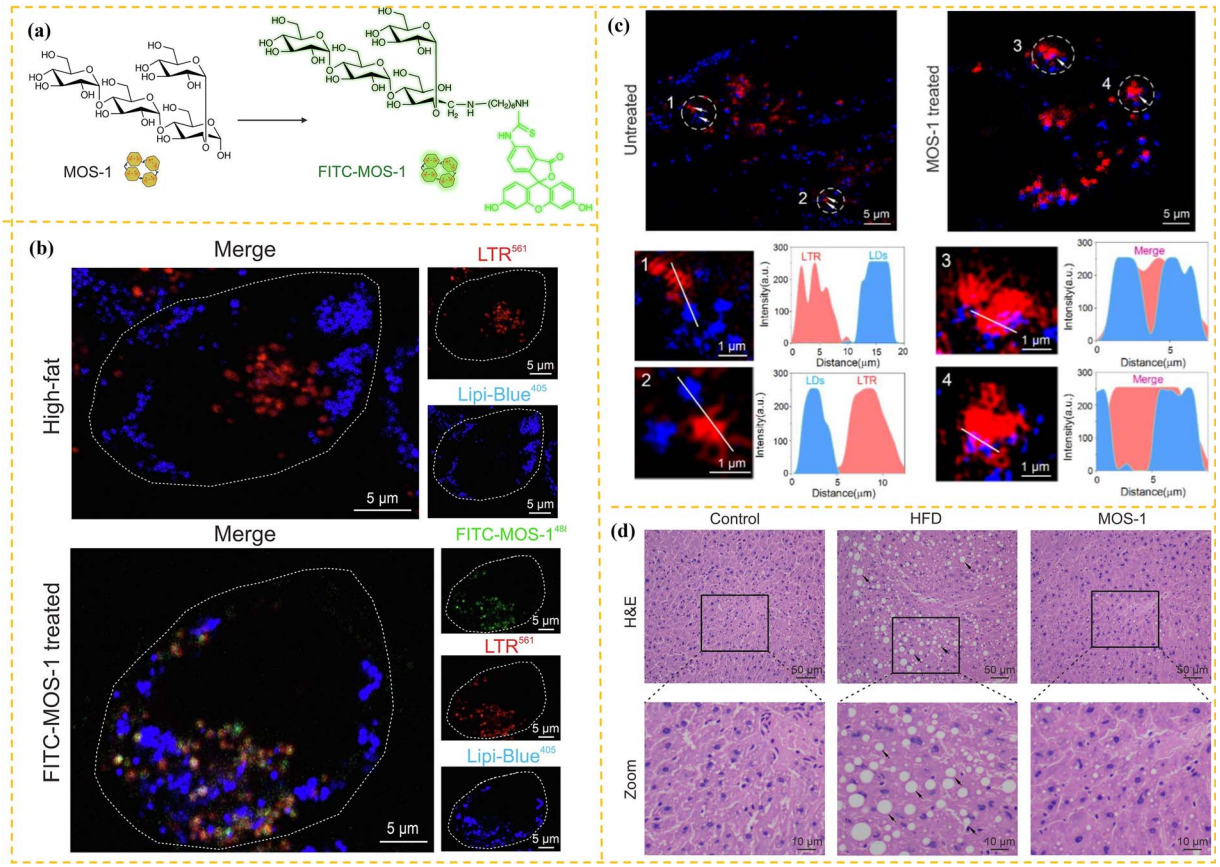

Fig. 43 (a) Chemical structures and reaction processes of compounds MOS-1 and **FITC-MOS-1**. (b) Fluorescence imaging of the interaction between lysosomes and lipid droplets during adipophagy in high-fat cells using **FITC-MOS-1**. (c) SIM imaging of the interaction between lysosomes and lipid droplets in cells treated and untreated with *Mytilus edulis* oligosaccharides (MOS-1). (d) Mussel oligosaccharides (MOS-1) reduce triglyceride staining imaging in a mouse model of fatty liver. Reprinted with permission from Elsevier, copyright 2024, as cited in ref. 104.

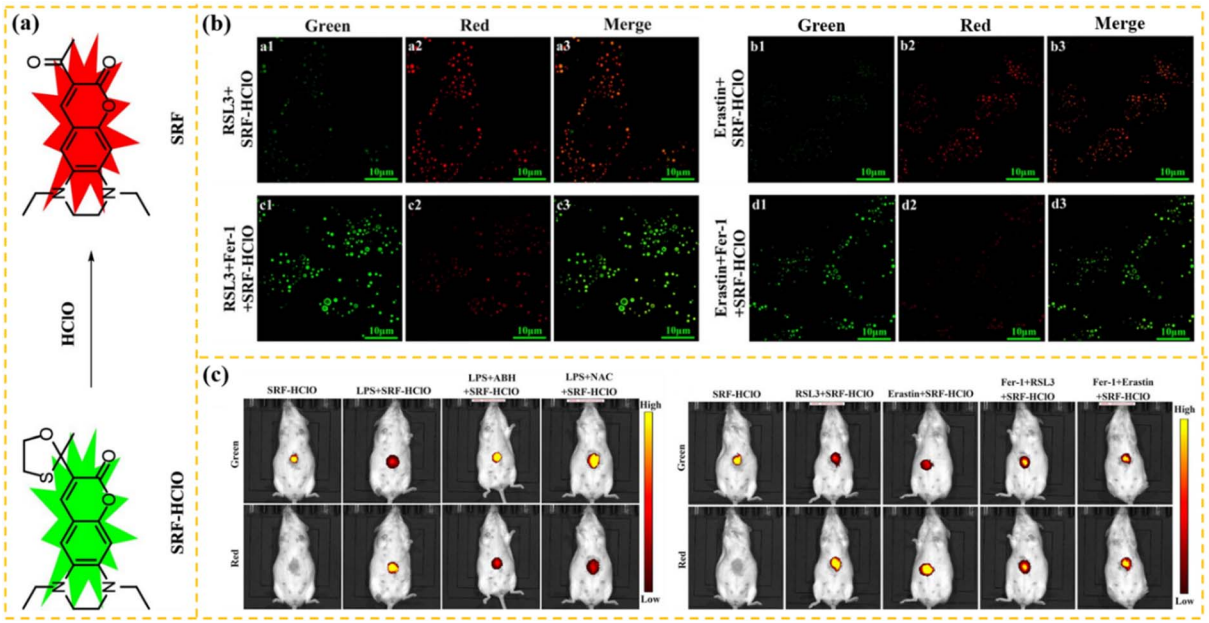

Fig. 44 (a) Chemical structure of probe **SRF-HClO** and the reaction mechanism of HClO. (b) Probe **SRF-HClO** is used for SIM imaging to detect cell HClO during different drug-induced ferroptosis processes. (c) SIM imaging of probe **SRF-HClO** in LPS-induced inflammation and different drug-induced ferroptosis mouse models. Reprinted with permission from American Chemical Society, copyright 2024, as cited in ref. 105.

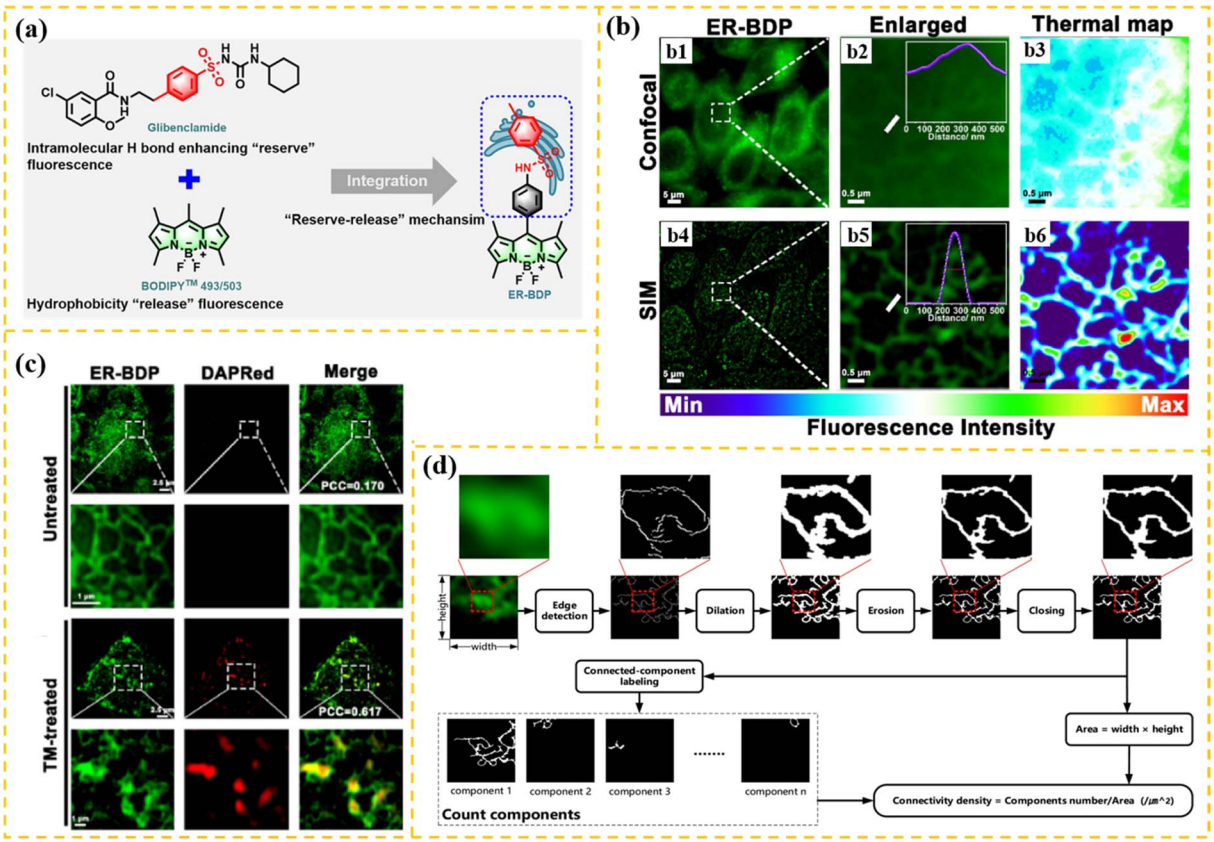

Fig. 45 (a) Design and chemical structure of probe **ER-BDP**. (b) Confocal imaging and SIM imaging of probe **ER-BDP** in HeLa cells. (c) SIM imaging of probe **ER-BDP** in the process of endoplasmic reticulum autophagy induced by streptomycin (TM). (d) The process of establishing algorithm-assisted topology tools and conducting reliability analysis. Reprinted with permission from Elsevier, copyright 2023, as cited in ref. 112.

probe has lysosome-targeting properties and can be used to study the interplay between lysosomes and other organelles in the process of fat phagocytosis. Meanwhile, oyster oligosaccharides MOS-1 can also be used to reduce triglyceride levels in adipocytes. In addition, the treatment process of MOS-1 on fatty liver mice was also studied, with results indicating that MOS-1 decreased the accumulation of TGs in the liver and improved liver function. This drug is expected to become a promising candidate for treating fatty liver. In conclusion, this article utilizes a fluorescence labeling strategy to reveal the distribution of MOS-1 within cells and its impact on lipid metabolism. However, a more detailed investigation into the specific mechanisms of MOS-1 action still requires further validation.

During that same year, Yang and colleagues designed a ratio-type fluorescent probe **SRF-HClO** for rapid detection of intracellular HClO.[105] The probe **SRF-HClO** can perform super-resolution imaging of the fine structure of lysosomes. It can also be effectively utilized for SIM imaging of lysosomal HClO in HeLa cells during ferroptosis (Fig. 44). In addition, the authors also used the probe **SRF-HClO** to detect the fluctuation of HClO levels in LPS-induced inflammatory mice and erastin induced iron death mouse models. This study is highly significant for evaluating the inhibitory effect of ferroptosis on solid tumors in mice. In summary, the **SRF-HClO** probe developed in this article enables super-resolution imaging of lysosomal HClO in ferroptotic cells. However, its investigation into the specific role of HClO in ferroptosis, as well as the probe's specificity and sensitivity validation in complex biological environments, remains insufficient and requires further in-depth study.

3.1.4 Fluorescent probes for endoplasmic reticulum SIM imaging. Among numerous organelles, the endoplasmic reticulum serves as an important biological structure within cells, responsible for various key functions such as protein synthesis, folding, transport, and calcium ion regulation. Its dynamic changes are intimately related to diverse physiological and pathological processes.[106–108] Although the endoplasmic reticulum plays an indispensable role in cells, traditional imaging methods cannot present its details due to its complex three-dimensional structure and interactions with other organelles.[109–111] Super-resolution imaging technology, especially structured light illumination microscopy (SIM), has opened up new horizons for endoplasmic reticulum research with its high resolution, low phototoxicity, and real-time imaging capabilities. The application of SIM technology combined with specific fluorescent probes greatly improves the imaging resolution of the endoplasmic reticulum, enabling researchers to observe the morphological changes and functional activities of the endoplasmic reticulum in real-time at the subcellular level,

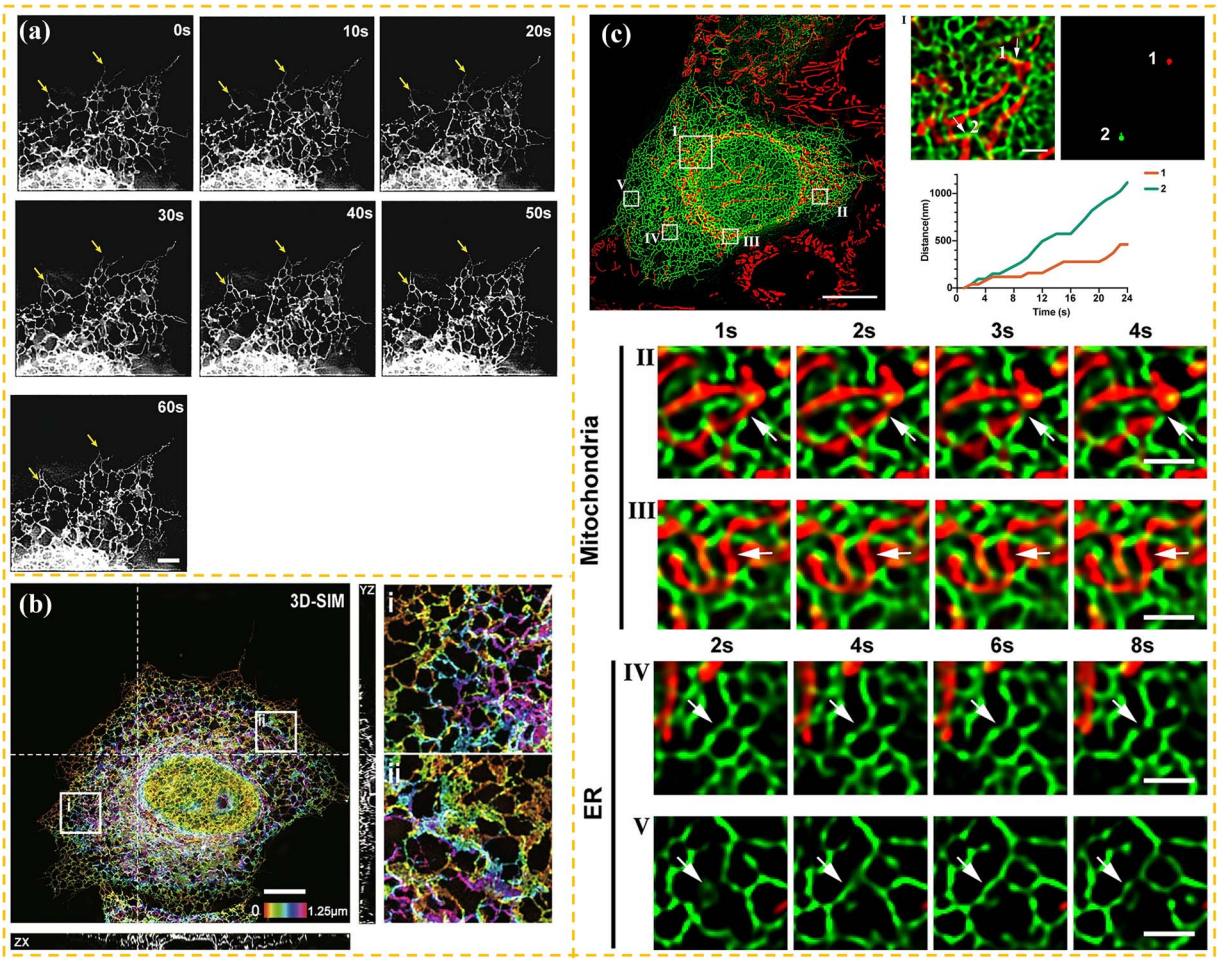

Fig. 46 (a) Delayed SIM imaging of the endoplasmic reticulum labeled by compound **RR-mNeonGreen** in HeLa cells. (b) Compound **RR-mNeonGreen** is used for 3D-SIM imaging of the endoplasmic reticulum in HeLa cells. (c) SIM imaging of the interaction process between the intracellular endoplasmic reticulum and mitochondria labeled with the commercial mitochondrial dye mitotracker using the compound **RR-mNeonGreen**. Reprinted with permission from Wiley, copyright 2023, as cited in ref. 113.

providing a powerful tool for further studying its functional mechanisms.

In 2023, Diao *et al.* designed and synthesized a fluorescent probe **ER-BDP** based on the BODIPY skeleton for super-resolution fluorescence imaging of ER.[112] As shown in Fig. 45, the probe **ER-BDP** can not only be used for imaging the fine structure of the endoplasmic reticulum. It can also be used to detect changes in the local hydrophobicity of the endoplasmic reticulum under different autophagy conditions. In addition, utilizing the hydrophobic properties of the probe **ER-BDP**, the authors also devised a topology analysis approach for real-time monitoring of changes in the endoplasmic reticulum structure. Importantly, this analytical tool can be used with other endoplasmic reticulum probes to analyze and detect endoplasmic reticulum autophagy processes. This work has broad application prospects for the quantitative detection and analysis of fine endoplasmic reticulum damage and stress. However, the in-depth analysis of the relationship between changes in ER hydrophobicity and disease progression is insufficient, and the probe's applicability and imaging specificity across different pathological states still require further validation.

In the same year, Ci and colleagues created a novel ER marker **RR-mNeonGreen** based on the green fluorescent protein mNeonGreen for super-resolution fluorescence imaging of ER.[113] Through super-resolution fluorescence imaging technology, **RR-mNeonGreen** facilitates continuous super-resolution imaging of ER tubules (Fig. 46). In addition, **RR-mNeonGreen** can also be used to study the dynamic processes of ER. The dynamic process of interaction between the endoplasmic reticulum and mitochondria was successfully observed. This work helps us understand the relevant mechanisms of endoplasmic reticulum dynamics. In summary, the **RR-mNeonGreen** probe introduced in this study greatly enhances the continuity and imaging resolution of the endoplasmic reticulum. However, understanding the detailed molecular mechanisms involved in its dynamic interactions with other organelles remains limited and needs further investigation.

In 2024, Zhang and colleagues created a gold nanocluster fluorescent probe **GNP-Atto565-fR8-CA** based on Halo labeling

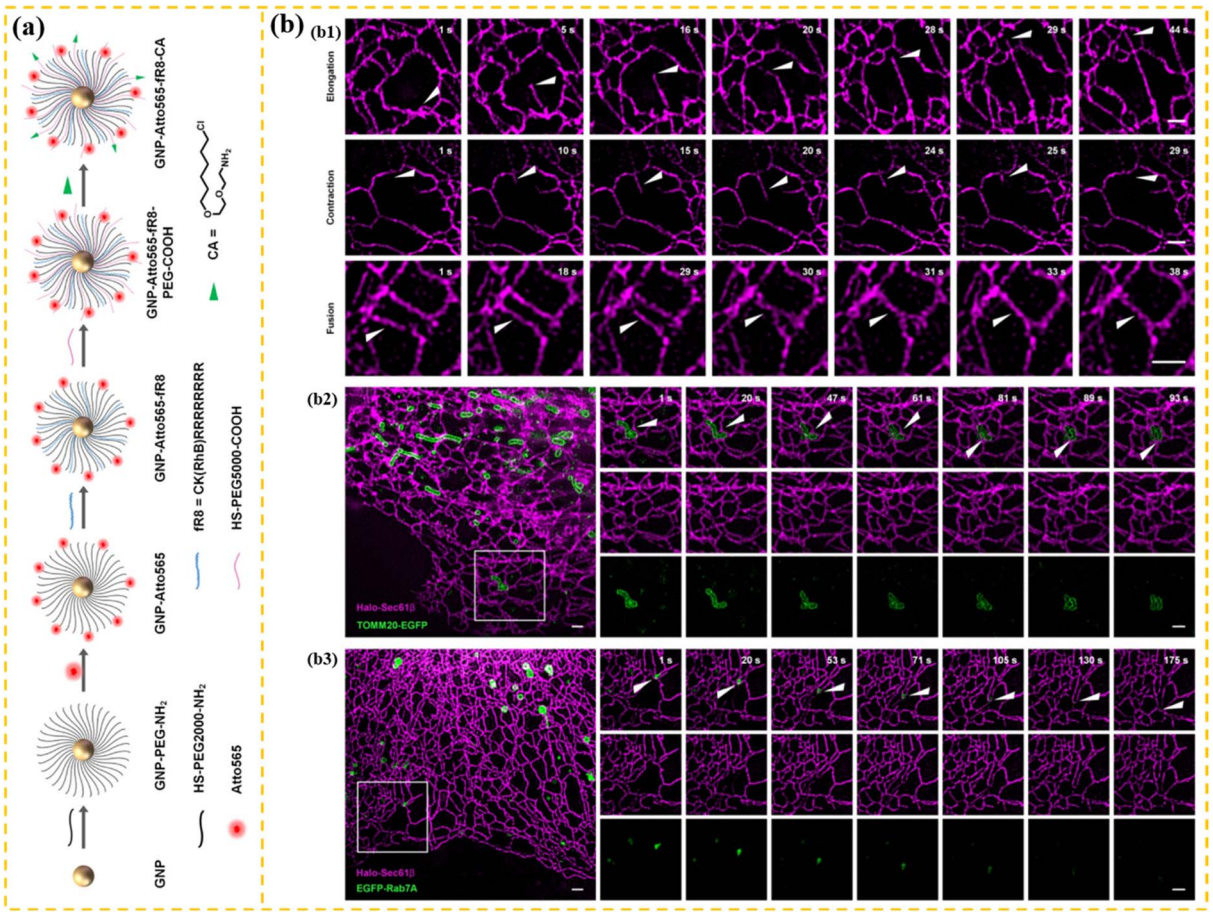

Fig. 47 (a) Schematic diagram of the synthesis process of probe **GNP-Atto565-fR8-CA**. (b) SIM imaging of probe **GNP-Atto565-fR8-CA** in U-2 OS cells. (b1) Delayed SIM imaging of probe **GNP-Atto565-fR8-CA** in U-2 OS cells. (b2) Dual color SIM imaging of probe **GNP-Atto565-fR8-CA** with Halo Sec61 β and TOMM20-EGFP in U-2 OS cells. (b3) Dual color SIM imaging of probe **GNP-Atto565-fR8-CA** with Halo Sec61 β and EGFP-Rab7A in U-2 OS cells. Reprinted with permission from American Chemical Society, copyright 2024, as cited in ref. 114.

for SIM imaging of the endoplasmic reticulum.[114] The probe comprises 3.5 nm GNPs modified with polyethylene glycol (PEG), fluorescent dye Atto565, cell-penetrating peptide fR8, and Halo tag ligand chloroalkane (CA). The probe **GNP-Atto565-fR8-CA** can efficiently load multiple Atto565 dye molecules, effectively delivering the probe into the cytoplasm, resulting in excellent photostability and biocompatibility. Through SIM imaging technology, **GNP Atto565-fR8-CA** can be used to study the dynamic processes of the endoplasmic reticulum in cells (Fig. 47). In addition, the probe can also be used to observe the process of interaction between the endoplasmic reticulum and other organelles. This probe offers a useful tool for visualizing the dynamic processes of the endoplasmic reticulum. Overall, the **GNP-Atto565-fR8-CA** probe presented in this study allows for high-brightness, extended super-resolution imaging of the dynamic endoplasmic reticulum. However, the molecular mechanisms driving these dynamic changes in the endoplasmic reticulum still need further detailed exploration.

3.1.5 Fluorescent probes for SIM imaging of cell membranes and vesicles. The cell membrane and vesicles are essential components in cellular function and signal transduction, with their interactions with organelles playing a critical role in biological research. As a boundary between cells and the external environment, the cell membrane has the dual characteristics of structural support and functional regulation. It not only plays a crucial role in material exchange, signal transmission, and cell recognition but also involves the formation and transportation of vesicles.[115–117] As an important component of the cell membrane, vesicles participate in the internalization, efflux, and intercellular interactions of substances. Studying the dynamic processes of cell membranes and their vesicles is of great significance for understanding the basic physiological mechanisms of cells and the mechanisms of related diseases.[118,119] SIM imaging reconstructs images from multiple angles, providing more detailed three-dimensional morphological information of cell membrane surfaces and vesicles than conventional microscopes, enabling clear visualization of dynamic changes in cell membranes and vesicle transport processes.[120] By utilizing SIM imaging technology, researchers can more accurately analyze the roles of cell membranes and vesicles in the transport of substances inside and outside the cell, revealing their precise regulation in

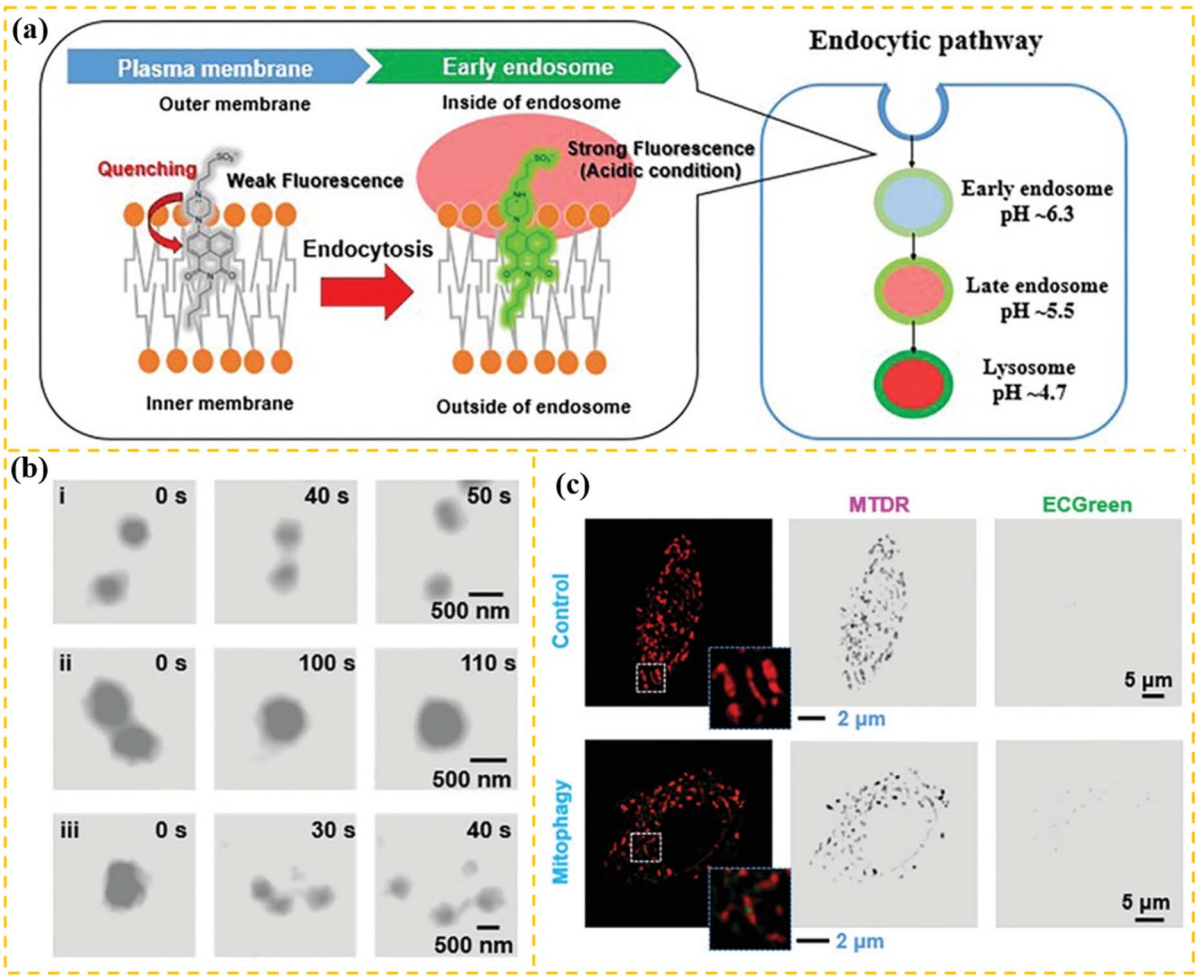

Fig. 48 (a) Simulation process of the chemical structure and endocytic pathway mechanism of probe **ECGreen**. (b) Dynamic delayed SIM imaging of probe **ECGreen** in HeLa cells. (c) Probe **ECGreen** tracks the delayed SIM imaging of the interaction between endocytic vesicles and mitochondria during cellular autophagy. Reprinted with permission from Wiley, copyright 2022, as cited in ref. 121.

cellular physiological activities. Specifically in disease research, SIM imaging can help us understand the relationship between abnormal vesicle transport and various diseases, providing an important basis for the development of related treatment strategies.

In 2025, Diao and colleagues created a membrane-anchored fluorescent probe **ECGreen** for detecting the pH of endocytic vesicles.[121] **ECGreen** is a novel small molecule fluorescent probe that integrates into the cell membrane through membrane anchoring properties. As shown in Fig. 48, the probe enters the cell through endocytosis, and its fluorescence signal progressively intensifies with the acidity within the vesicle environment, breaking through the limitations of traditional acidic vesicle probes. Due to the probe being firmly attached to the inner wall of the vesicle, its fluorescence intensity is not affected by pH fluctuations, effectively resisting the erosion of reactive substances inside the vesicle, greatly improving its stability and resistance to photobleaching during long-term tracking of endocytosis. Based on the unique properties of **ECGreen**, the author also constructed a multidimensional analysis framework that integrates space, time, and pH values, providing a new tool for the systematic study of endocytosis kinetics. Using this model, the authors also successfully revealed the information transmission, migration behavior, and spatial distribution of phagocytic vesicles in living cells, and deeply discussed their interaction with mitochondria during autophagy. At the same time, they observed the rapid dynamic transformation of endosomes around the plasma membrane. This work significantly improves the diagnostic level of diseases and provides an important basis for studying subcellular dynamics processes. The **ECGreen** probe introduced in this study allows high-resolution imaging of endosomes *via* membrane anchoring and pH-responsive characteristics. However, the exploration of the molecular mechanisms behind the dynamic changes in endosomes and their interactions with other organelles remains limited. Furthermore, the probe's general applicability across various cell types and physiological or pathological conditions has not been extensively validated.

In 2023, Xu *et al.* designed and developed a visual fluorescent probe that encodes the receptor binding domain (RBD) of viral

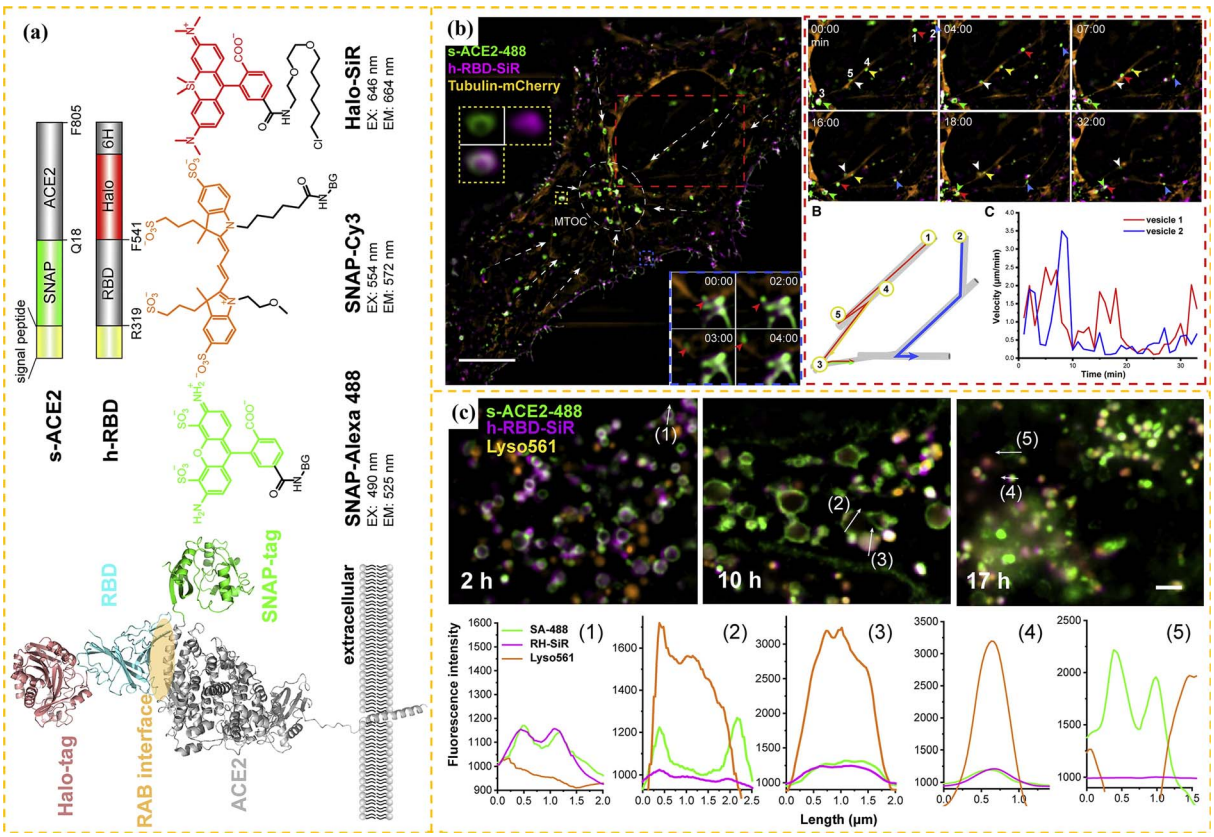

Fig. 49 (a) Schematic diagram of the design of RAB fusion protein, s-ACE2 and h-RBD constructs, and chemical structure of the Halo/SNAP probe. (b) SIM imaging of compound **s-ACE2-488/h-RBD-SiR** tracking the movement and maturation of vesicles in HeLa cells. (c) SIM imaging of the degradation process of compounds s-ACE2 and h-RBD in HeLa cells. Reprinted with permission from Elsevier, copyright 2023, as cited in ref. 122.

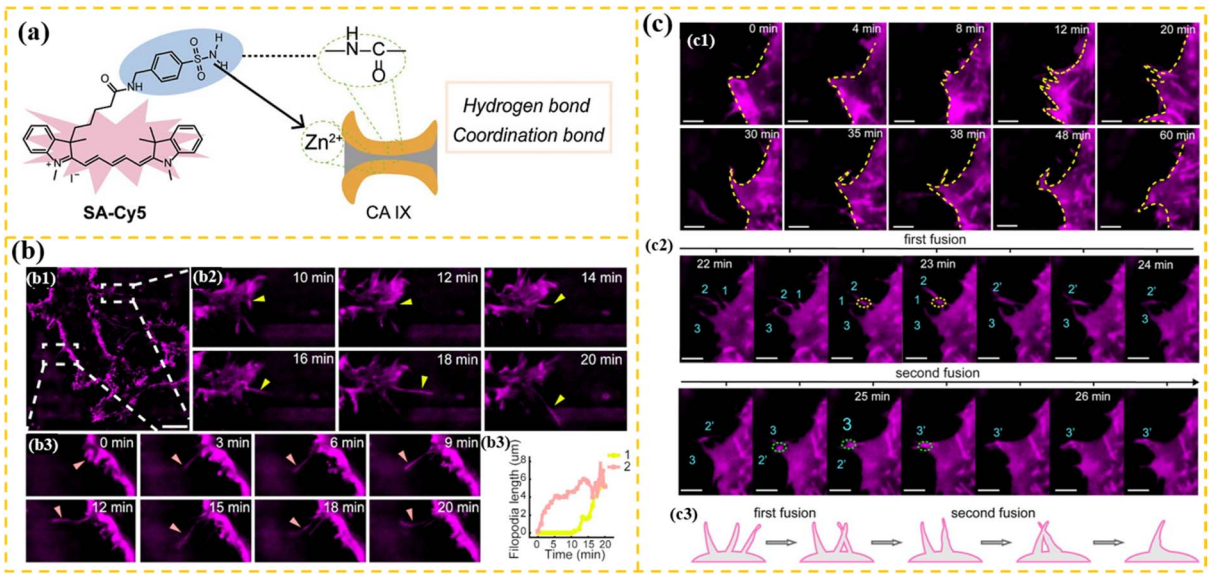

Fig. 50 (a) Response mechanism of probe **SA-Cy5** specifically recognizing carbonic anhydrase IX. (b) SIM imaging of the **SA-Cy5** probe for monitoring the membrane dynamic growth and filamentous pseudopodia fusion process in HeLa cells. (c) Probe **SA-Cy5** monitors the delayed SIM imaging process of vesicle fusion in HeLa cells. Reprinted with permission from American Chemical Society, copyright 2024, as cited in ref. 123.

spike proteins and angiotensin-converting enzyme 2 (ACE2) in airway epithelial cells using Halo and SNAP protein tags, and the probe was labeled with rhodamine and cyanine dyes to monitor RAB and endocytosis in live cells.[122] Rhodamine and cyanine dyes have excellent photostability, and the endocytosis of RBD can be solved by imaging the position and ratio of ACE2/RBD fluorescence (as shown in Fig. 49). In addition, the author not only tracked the translocation and maturation process of ACE2/RBD co-localization vesicles through SIM imaging, but also showed the process of RBD and ACE2 proteins being absorbed and degraded by lysosomes. This study provides a valuable tool for understanding the mechanism of SARS-CoV-2 infection. Overall, the innovation of this study lies in the integration of various dye types and protein tags, which significantly enhance image resolution and the signal-to-noise ratio, providing a reliable tool for dynamic monitoring of virus–receptor interactions in live cells. This study offers a detailed examination of the endocytosis process of SARS-CoV-2 RBD using SIM technology. However, additional experiments are required to investigate critical steps, including how the RBD–ACE2 complex mediates the release of viral RNA.

Subsequently, the team designed and synthesized a cyanine dye **SA-Cy5** based on a buffering strategy for specific binding to carbonic anhydrase IX in the cell membrane.[123] This probe can overcome photobleaching and has excellent photostability. As shown in Fig. 50, by SIM imaging technology, the probe **SA-Cy5** can image the cell membrane for a long time. In addition, by using the probe **SA-Cy5**, the authors also observed the dynamic process of growth and fusion of filamentous pseudopodia on the cell membrane. This work provides potential insights for studying cell dynamics. This article demonstrates the potential of the **SA-Cy5** probe for nanoscale dynamic imaging of the cell membrane. However, it does not provide a comprehensive investigation into how dynamic membrane changes affect cellular physiological functions. Moreover, the experimental conditions are somewhat restricted, and the probe's efficacy in varied and complex environments has not been thoroughly evaluated.

In the same year, the team also reported a series of buffered fluorescent probes **BMP** for nanoscale imaging of the plasma membrane (PM).[124] Among them, **BMP-14** and **BMP-16** have buffering ability, exhibit excellent photostability, and can label PM for a long time. As shown in Fig. 51, the authors used the probe **BMP** to monitor the dynamic movement and sustained contraction process of PM filamentous pseudopodia through SIM imaging. In addition, they also observed the fusion process of two different extracellular vesicles. In addition, the probe **BMP-16** exhibits a unique ability to induce single-molecule fluorescence scintillation, making it suitable for rapid fluorescence imaging of cell membranes. The **BMP-14** and **BMP-16**

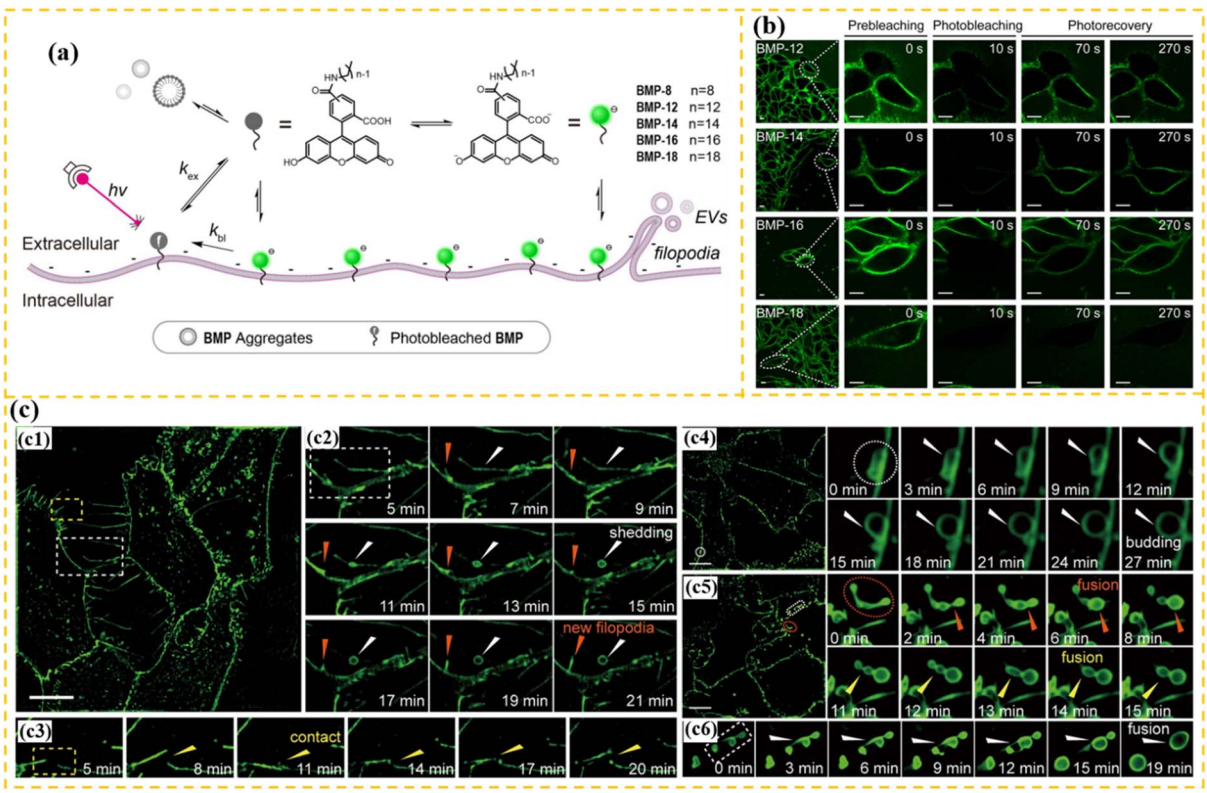

Fig. 51 (a) A schematic illustration depicting the chemical architecture of probe **BMP** and the simulation of the labeled cell membrane. (b) Delayed buffered SIM imaging of compound **BMP** in HeLa cells. (c) Dynamics SIM imaging of compounds **BMP-14** and **BMP-16** in the HeLa cell membrane. Delayed SIM imaging of the plasma membrane of (c1–c3) compound **BMP-14** in HeLa cells. Long-term SIM imaging of the plasma membrane in HeLa cells using compound **BMP-16** (c4–c6). Reprinted with permission from American Chemical Society, copyright 2024, as cited in ref. 124.

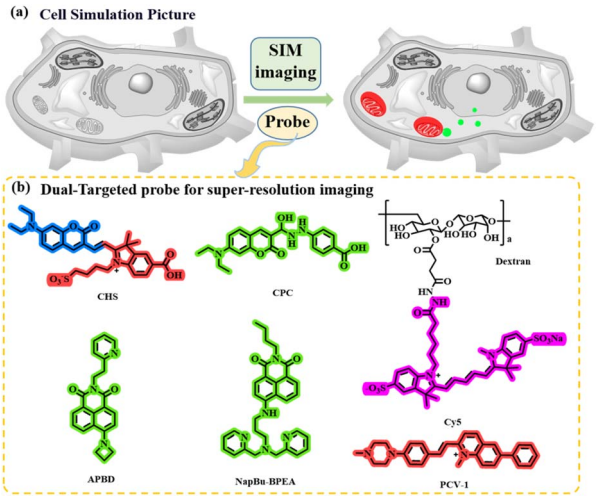

Fig. 52 Approaches to the design of dual-targeted fluorescent probes. (a) Computational design of fluorescent probes for dual cellular targeting. (b) Reported fluorescent probes for SIM imaging.

probes developed in this article successfully enable super-resolution imaging of cell membrane morphology. However, the article provides limited insight into the mechanisms by which the probes specifically influence or reveal membrane-related biological processes. Additionally, the experiments focus primarily on specific cell types, lacking a comprehensive assessment of the probe's performance across different cell types and physiological or pathological conditions.

3.1.6 Fluorescent probes for multi-organelle SIM imaging. As the fundamental unit of cellular function, organelles play a crucial role in cellular life activities. However, organelles do not exist in isolation, but rather work together through complex interaction networks to complete various biological processes. Studying the dynamic interactions of multiple organelles is of great significance for revealing cellular physiology and pathological mechanisms.[125,126] Traditional single organelle-targeted fluorescent probes can only label and observe specific organelles, making it difficult to fully reveal the synergistic relationships between multiple organelles. Therefore, the development

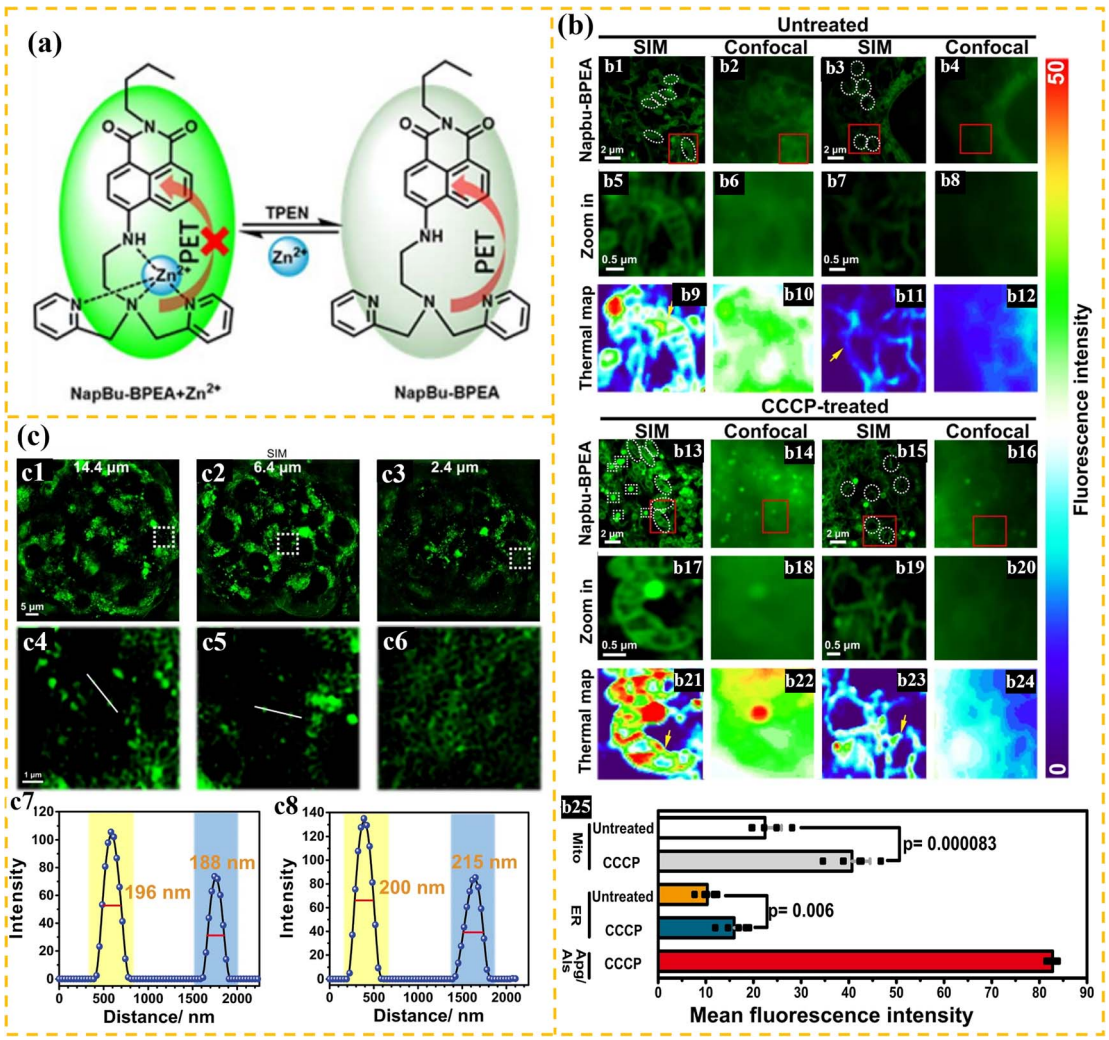

Fig. 53 (a) Reaction process of probe **NapBu-BPEA** for detecting Zn^{2+}. (b) SIM imaging of Zn^{2+} detection in mitochondria, autophagosomes, and the endoplasmic reticulum using the CCCP induced autophagy process probe **NapBu-BPEA**. (c) SIM imaging of the **NapBu-BPEA** probe for recognizing organelles in liver organoids. Reprinted with permission from Nature, copyright 2021, as cited in ref. 131.

of fluorescent probes that can simultaneously target multiple organelles has become a research hotspot in recent years. This type of probe achieves selective labeling of multiple organelles (such as mitochondria, the endoplasmic reticulum, lipid droplets, lysosomes, *etc.*) through clever molecular design, and uses multi-channel fluorescence imaging technology for synchronous observation, providing a powerful tool for studying organelle interaction[127] (Fig. 52). In recent years, by combining fluorescent probes that simultaneously target multiple organelles with SIM imaging technology, researchers have been able to reveal key regulatory mechanisms of organelle interactions in cellular metabolism, autophagy, disease occurrence, and other processes with higher spatiotemporal resolution.[128–130] This study is of great significance for understanding cellular homeostasis regulation, disease occurrence and development, and drug development.

In 2021, Diao *et al.* designed and synthesized a fluorescent probe **NapBu-BPEA** based on 1,8-naphthalimide as a fluorescent group for the detection of Zn^{2+} in living cells.[131] This probe can simultaneously target multiple organelles including lysosomes, mitochondria, and the endoplasmic reticulum, and can also be used for reversible detection of intracellular Zn^{2+}. In addition, through SIM imaging, the probe **NapBu-BPEA** can also be used to monitor the changes in Zn^{2+} in mitochondria, lysosomes, and the

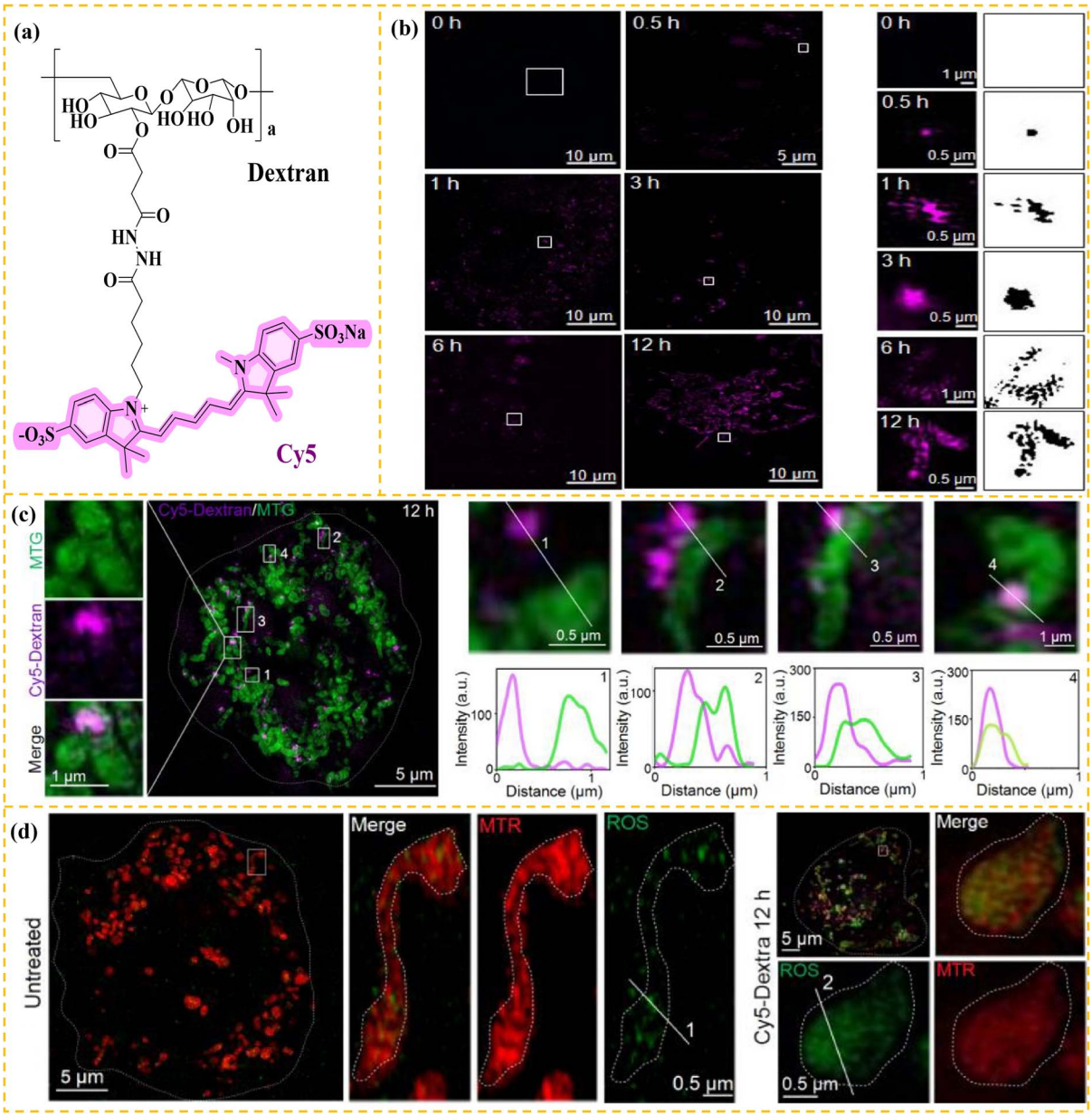

Fig. 54 (a) Chemical structure of probe **Cy5-Dextran**. (b) SIM imaging of the **Cy5-Dextran** probe targeting organelles in HeLa cells at different time periods. (c) SIM imaging of HeLa cell probe **Cy5-Dextran** in contact with mitochondria and mitochondrial fragmentation. (d) SIM imaging of the **Cy5-Dextran** probe and ROS commercial green probe for monitoring ROS levels in HeLa cells. Reprinted with permission from Elsevier, copyright 2022, as cited in ref. 132.

endoplasmic reticulum during CCCP-induced autophagy (Fig. 53). Most importantly, the probe has also been successfully used for SIM imaging to identify organelles in liver organoids. This study provides important evidence for understanding the biology related to Zn^{2+} in complex biological models. The Zn-STIMO method presented in this article enables simultaneous tracking of Zn^{2+} across multiple organelles. However, the article provides insufficient discussion on how fluctuations in Zn^{2+} specifically regulate organelle functions and cellular fate. Furthermore, the experiments are primarily based on the HeLa cell model, and the applicability to other cell types requires further validation.

In 2022, Chen and colleagues created a **Cy5-Dextran** fluorescent probe for SIM imaging of cellular lysosomes and mitochondria based on the visual therapeutic diagnostic mechanism of nanoscale pectin *in vivo*.[132] This probe can exhibit time-dependent distribution of lysosomes and mitochondria. When the probe is incubated with cells for 1–3 hours, the cells are distributed in lysosomes. When incubated for 6–12 hours, **Cy5-Dextran** needles were found to be localized in mitochondria and could induce mitochondrial damage processes (Fig. 54). In addition, the author also investigated how increasing ROS levels within cells can accelerate mitochondrial fragmentation and promote the process of cancer cell apoptosis. This article utilizes SIM technology to reveal the subcellular distribution of dextran in live cells. However, it primarily focuses on morphological observations, with insufficient exploration of the molecular mechanisms by which dextran exerts its antitumor bioactivity through the regulation of organelle functions.

In the same year, the group designed and developed a fluorescent probe **CHS** grounded in the coumarin hemicyanine structure for detecting RSS in intracellular mitochondria and lysosomes.[133] This probe can be used for visualizing RSS of mitochondria and lysosomes within live cells, with fluorescence observed in the blue and red channels, respectively (Fig. 55). In addition, the RSS crosstalk of **CHS** in the interaction zone between cell mitochondria and lysosomes was also studied. Through experiments, it was found that the probe **CHS** can target lysosomes induced by mitochondrial RSS and be transferred through MLC sites. In summary, the CDtSC strategy and **CHS** probe introduced in this study offer an innovative method for single-image visualization of RSS exchange at mitochondria–lysosome contact points. However, the article lacks a detailed analysis of the molecular mechanisms by which RSS exchange specifically affects organelle functions and cellular outcomes. Moreover, the broader applicability and effectiveness of this method across different physiological and pathological conditions still need further confirmation.

Subsequently, Park *et al.* designed and developed a series of highly photostable NTD probes (**NTD-Mor**, **NTDT-Mor**, **NTDT-TPP**, and **NTDT-DCV-TPP**) based on 1,5-naphthalene-2,6-dione (NTD) as a fluorescent scaffold for multi-color and super-resolution fluorescence imaging in live cells.[134] This series of probes has good biocompatibility and can target mitochondria and lysosomes. Compared with commercial dyes, it has excellent photostability. In addition, this series of probes has been effectively utilized for organelle-specific multi-color imaging and super-resolution imaging of CLSM (Fig. 56). This work offers an important method for developing more novel super-resolution fluorescent probes. Although this article demonstrates the excellent photostability of NTD-based fluorescent probes for organelle imaging, it does not provide a thorough examination of the mechanisms behind their interactions with organelles or

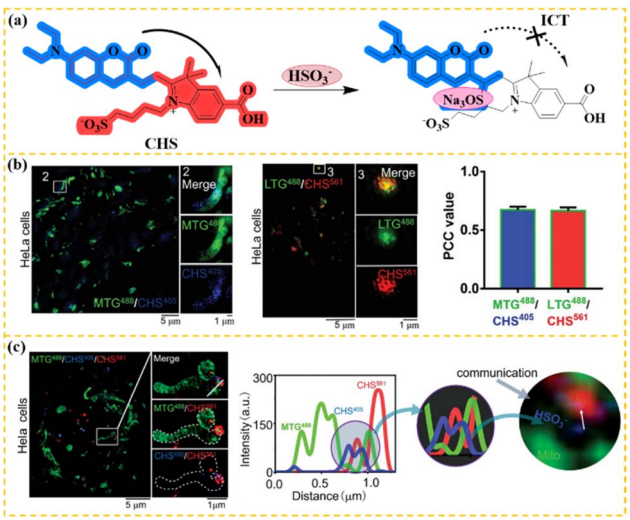

Fig. 55 (a) Reaction mechanism of probe **CHS** detection of HSO_3^-. (b) SIM imaging of co-localization of mitochondria and lysosomes in HeLa cells using probe **CHS**. (c) SIM imaging of RSS crosstalk in the interaction process between mitochondria and lysosomes in HeLa cells using probe **CHS**. Reprinted with permission from Wiley, copyright 2022, as cited in ref. 133.

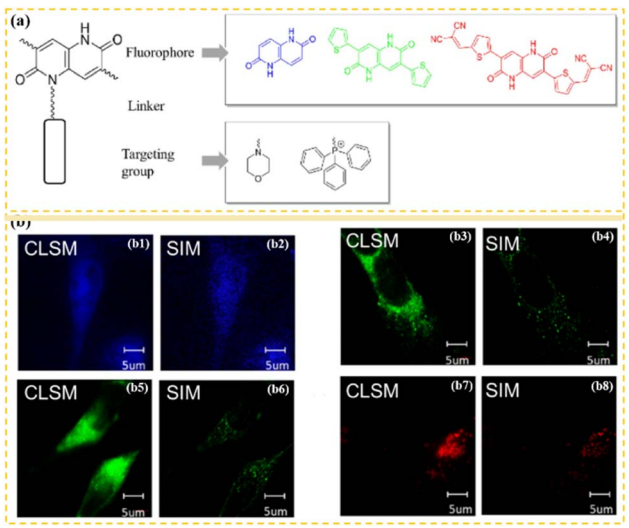

Fig. 56 (a) Design of the NTD probe and organelle-specific targeting groups. (b) Confocal imaging and SIM imaging of NTD probes (**NTD-Mor**, **NTDT-Mor**, **NTDT-TPP**, and **NTDT-DCV-TPP**) in HeLa cells. (b1 and b2) **NTD-Mor**. (b3 and b4) **NTDT-Mor**. (b5 and b6) **NTDT-TPP**. (b7 and b8) **NTD-DCV-TPP**. Reprinted with permission from Elsevier, copyright 2022, as cited in ref. 132.

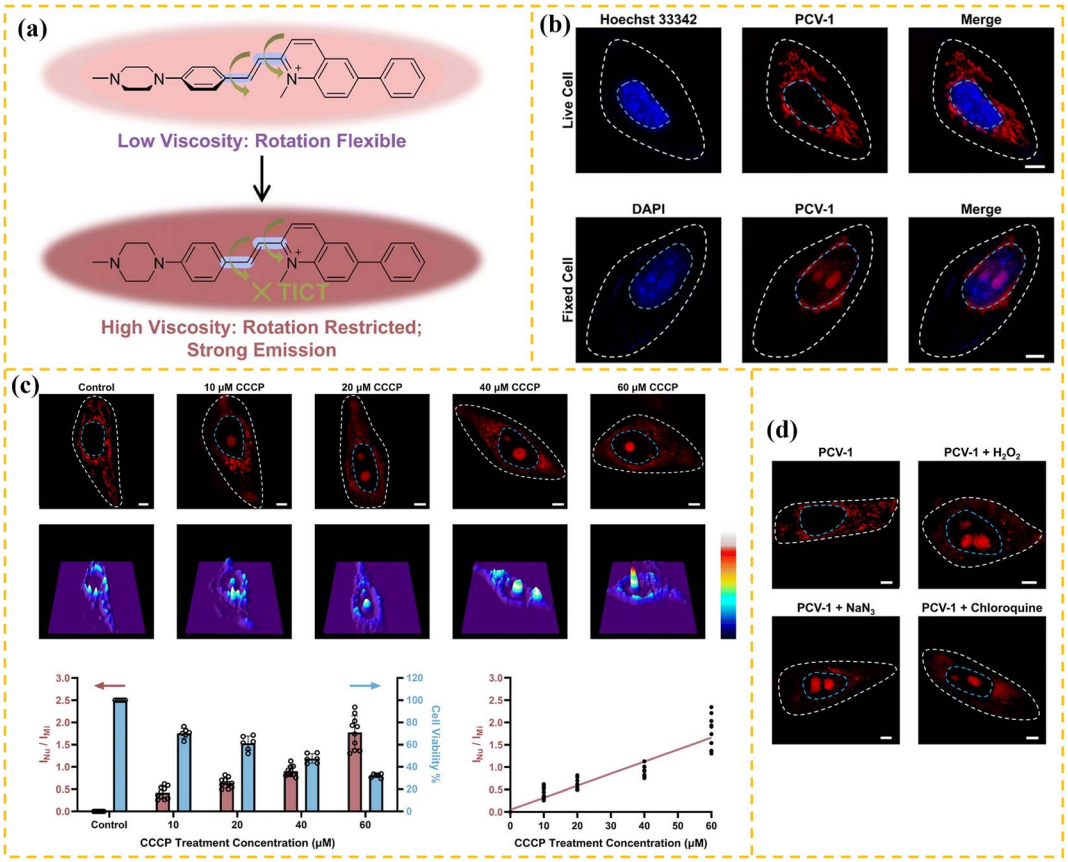

Fig. 57 (a) Reaction process of probe **PCV-1** in response to viscosity. (b) SIM imaging of probe **PCV-1** in live and fixed cells. (c) SIM imaging of cell viability monitored by the **PCV-1** probe during CCCP-induced autophagy in live cells. (d) Using ORP analysis to monitor SIM imaging of cell viability during the cell death process. Reprinted with permission from Royal Society of Chemistry, copyright 2022, as cited in ref. 136.

their long-term biotoxicity. Additionally, the potential applications and dynamic imaging capabilities of these probes need further validation and expansion.

Cell viability assays play a key role in studies such as precancer abnormal cell discovery, therapy evaluation and drug toxicity testing.[135] Therefore, Diao and colleagues created a fluorescent probe **PCV-1** for SIM imaging to detect cell viability.[136] As shown in Fig. 57, the probe is sensitive to mitochondrial membrane potential and has a strong affinity for DNA, making it suitable for staining mitochondria and nucleoli within cells. In addition, through SIM imaging, the migration of probe **PCV-1** from mitochondria to nucleoli during cell damage can be observed. Finally, the authors also developed an organelle ratio detection analysis method (ORP) combined with the probe **PCV-1** for quantitative analysis and evaluation of single-cell viability. This probe paves the way for quantitative analysis and detection of single-cell viability levels. While this article introduces an innovative method for quantifying cell viability using the **PCV-1** probe, it offers limited insight into the molecular mechanisms governing probe migration during cell death. Moreover, the experiments are mainly conducted on a specific cell line, and the broader applicability of the method needs further validation.

In 2024, Shao *et al.* designed and developed a fluorescent probe **CPC** based on coumarin as the fluorescent matrix for SIM imaging of intracellular organelles.[137] The probe **CPC** can simultaneously monitor the polarity changes of $ONOO^-$ and lipid droplets within mitochondria. In addition, the probe has been successfully used for SIM imaging of the interaction between lipid droplets and mitochondria during the process of ferroptosis. During erastin induced ferroptosis, the contact between mitochondria and lipid droplets decreased, and their PCC decreased from 0.53 to 0.26, indicating that erastin promoted the uncoupling of LDs with mitochondria (Fig. 58). While this study introduces the **CPC** dual-response probe for monitoring organelle dynamics, the investigation into the probe's response mechanism at the molecular level, as well as its applicability across different physiological and pathological states, remains limited. Further exploration of its potential applications is needed.

In the same year, Zhang *et al.* developed a novel nano-embedded fluorescent probe **FNPs-fR8-HTL** for SIM imaging of intracellular organelles in living cells.[138] By combining this probe with fusion protein technology, FNPs have achieved high specificity and stability imaging of living cell organelles. Through SIM imaging, the probe can also be used to observe the dynamic processes of the endoplasmic reticulum in cells. In

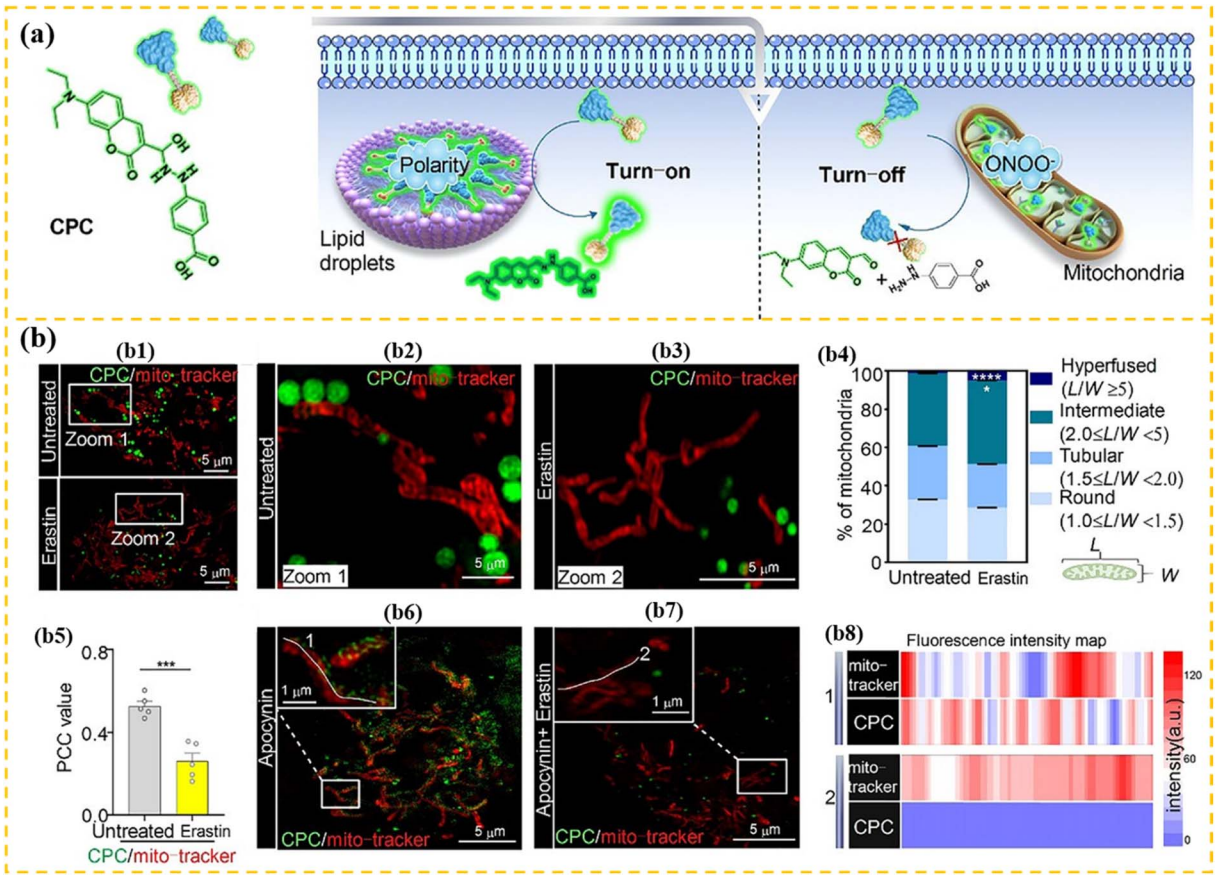

Fig. 58 (a) A schematic representation showcasing the chemical architecture of probe **CPC** and bimodal detection of mitochondrial $ONOO^-$ and lipid droplet polarity. (b) Fluorescence changes of mitochondria and lipid droplets during cell iron death and SIM imaging of interactions between mitochondria and lipid droplets. (b1) SIM imaging of probes and mitochondrial commercial dye (PKMTDR) in cells induced by erastin or without erastin induction. (b2 and b3) Magnified regions of Zoom 1 and Zoom 2 in (b1). (b4) Quantitative analysis of mitochondria in or out of erastin-induced mitochondria maps. (b5) Quantitative analysis plots of mitochondrial and lipid droplet co-localization under or without erastin induction. (b6 and b7) SIM imaging of cells stained with probe **CPC** with PKMTDR in or out of erastin-treated apocynin-induced cells. (b8) Plots of the fluorescence intensity distribution in extended white lines 1 and 2 in (b6) and (b7). Reprinted with permission from Wiley, copyright 2024, as cited in ref. 137.

addition, the interaction process between ER and mitochondria was successfully visualized using the probe **FNPs-fR8-HTL** (as shown in Fig. 59). This probe exhibits significant potential for utilization in prolonged super-resolution imaging applications. This study not only addresses the delivery bottleneck of FNPs in live-cell imaging but also demonstrates their significant potential in cell biology research and disease mechanism exploration. While this article highlights the efficiency of the cell-penetrating peptide fR8 in facilitating the cytoplasmic delivery of fluorescent nanoprobes, the detailed mechanisms of its interaction with the cell membrane remain to be explored.

In 2025, our group designed a water-sensitive probe **APBD** based on naphthalimide as a fluorescent scaffold for multi-organellar SIM imaging.[139] This probe has excellent photostability, high cell permeability, and wash-free properties, making it suitable for long-term imaging within cells. In this work, by utilizing the probe **APBD**, we observed three stages of mitochondrial morphological changes during cell necrosis (including swelling, division, and fusion). It was also successfully detected that the morphological changes in mitochondria can lead to the rupture of the endoplasmic reticulum, followed by the reconstruction of autophagosomes at the end of the endoplasmic reticulum tubules (as shown in Fig. 60). This study provides guidance for the development of more novel lipid membrane probes and is of great significance for studying different biological fields. Overall, this article presents the development of an exchangeable SIM fluorescent probe, **APBD**, which enables real-time monitoring of organelle dynamics and intracellular water distribution in necrotic cells. This advancement offers a novel and powerful tool for understanding the mechanisms of cell death and intracellular water dynamics, showcasing its broad potential applications in cell biology and disease research. While this article introduces a novel strategy using the **APBD** probe to track organelle dynamics and intracellular water heterogeneity in necrotic cells, its applicability across various physiological and pathological conditions remains inadequately validated. Additionally, long-term imaging effectiveness and biosafety need further assessment.

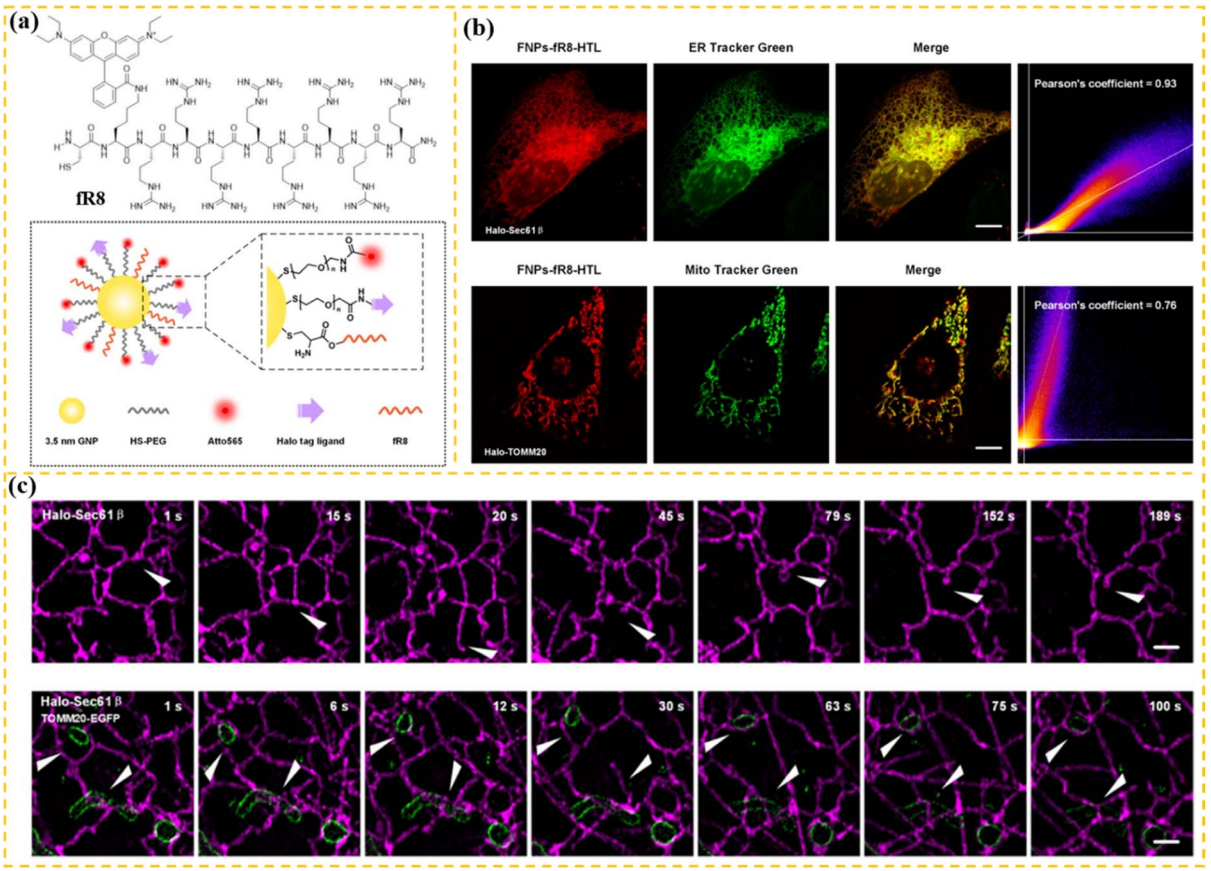

Fig. 59 (a) Chemical structure of the peptide sequence fR8 and design process of nanoprobes **FNPs-fR8-HTL**. (b) Co localization imaging of probe **FNPs-fR8-HTL** in U-2OS cells. (c) Delayed SIM imaging of probe **FNPs-fR8-HTL** in U-2OS cells and SIM imaging of mitochondrial endoplasmic reticulum interaction. Reprinted with permission from American Chemical Society, copyright 2024, as cited in ref. 138.

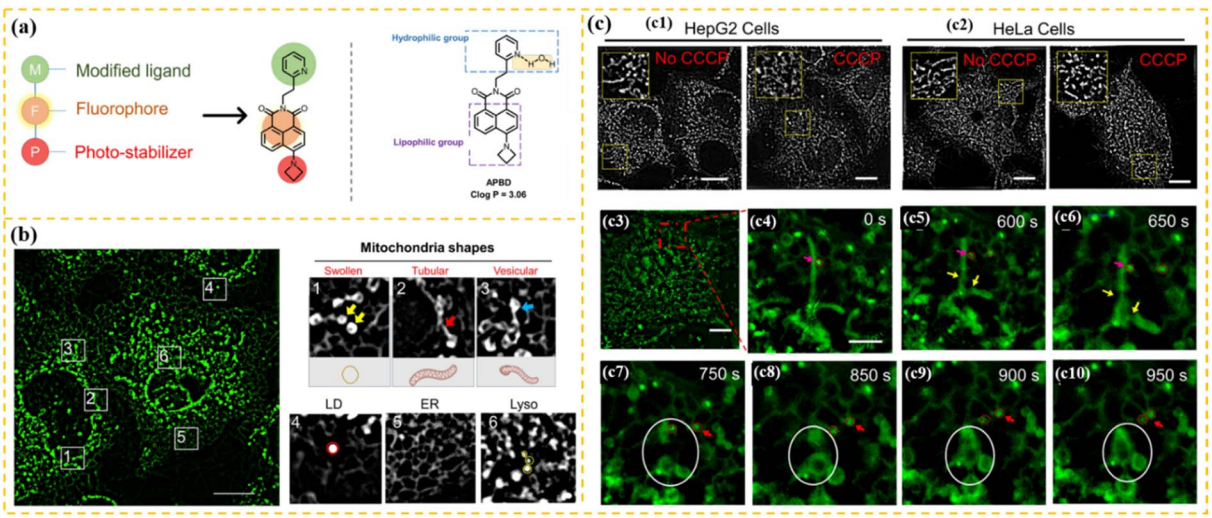

Fig. 60 (a) Design process and chemical structure of probe **APBD**. (b) SIM imaging of probe **APBD** labeling multiple organelles in HeLa cells. (c) Probe **APBD** is used to track SIM imaging of multiple organelles and membrane contact imaging between different organelles during the necrosis process in CCCP-induced necrotic cells. Reprinted with permission from PNAS, copyright 2025, as cited in ref. 139.

4. Conclusions and outlook

The combination of organelle-targeted fluorescent probes and structured light illumination microscopy (SIM) technology has brought revolutionary breakthroughs to the field of organelle super-resolution imaging. Through specific labeling strategies such as fusion protein labeling, small molecule ligand modification, or nanomaterial loading, the probe achieves precise

localization of subcellular structures such as mitochondria, the endoplasmic reticulum, lipid droplets, lysosomes, *etc.* Its high brightness, photostability, and low background noise characteristics form a synergistic effect with the high spatiotemporal resolution advantage of SIM. Research has shown that such probes not only significantly improve the capture accuracy of organelle dynamics processes (such as millisecond-level events of mitochondrial fission/fusion), but also reveal organelle interaction networks through multi-color imaging techniques (such as the contact mechanism between mitochondria and lipid droplets, mitochondria and lysosomes), providing a new molecular perspective for subcellular functional research. In summary, the advancement of organelle-targeted fluorescent probes for super-resolution SIM imaging shows great potential in advancing our understanding of cell structure and dynamics.

Although small molecule fluorescent probes have made breakthrough progress in the field of super-resolution structured light illumination microscopy (SIM) imaging, their technical potential and application boundaries still need to be deeply expanded. From the current research progress, there is still room for optimization in the core performance of probes, such as targeting specificity, photon conversion efficiency, and *in vivo* compatibility. However, their application value in complex scenarios such as organelle interaction network analysis and subcellular dynamic process tracking has not been fully released. In addition, as a powerful super-resolution imaging technology, SIM has some limitations despite its advantages of fast imaging and low phototoxicity. Firstly, the resolution of SIM is typically around 100–150 nm. Compared to technologies such as STED, SMLM, and STORM, its resolution is lower, making it difficult to meet the requirements for extremely high resolution, especially when studying molecular level structures, which may not be precise enough. Secondly, SIM has high requirements for fluorescence labeling of samples, and improper labeling or poor fluorescence molecular weight can affect imaging quality. In addition, SIM technology relies on image reconstruction, and although the imaging speed is fast, it still requires powerful computing power when processing large-scale data, which may become a bottleneck in the data analysis process. In view of this, in order to systematically promote the iterative upgrading and cross-border application of super-resolution fluorescence imaging technology, it is recommended to focus on breakthroughs in the following areas:

(1) Probe performance optimization: future fluorescent probes will further improve sensitivity, provide a higher signal-to-noise ratio, more effectively detect low abundance cellular components, and more accurately track the dynamic changes in organelles over time. In addition, to improve the practicality of super-resolution SIM imaging, the following measures can be taken: introducing probes with enhanced resistance to photobleaching by incorporating groups with strong electronic effects; adding antioxidants or other protective molecules to slow down the photodegradation process; selecting appropriate excitation conditions to avoid overexposure; developing new fluorescent probes with higher photostability, such as quantum dots or novel organic dyes; and utilizing nano-encapsulation and photoprotective materials to reduce photodamage. These technological innovations will support the implementation of efficient imaging over longer time spans, providing critical technical support for deeper probing of long-term cellular processes.

(2) Targeted strategy innovation: future probes will enhance their targeting ability by increasing affinity for specific organelles and subcellular compartments, reducing off-target effects and improving spatial resolution and imaging accuracy. Therefore, by incorporating various subcellular targeting moieties, efficient fluorescent probes will be developed to target different organelles (such as vesicles, the endoplasmic reticulum, lysosomes, *etc.*), enabling precise labeling and dynamic monitoring of subregions within organelles, such as mitochondrial cristae membrane proteins;

(3) Multi-targeted design strategy: in recent years, there has been increasing interest in the development of multimodal probes capable of simultaneously targeting multiple organelles. These probes can provide complementary imaging information, enabling multidimensional analysis of organelles and their interactions. The application of such probes will significantly advance the comprehensive study of molecular interactions and cellular dynamics. Therefore, by incorporating multiple targeting groups within a single molecular framework, the development of fluorescent probes with multifunctional targeting abilities will not only help reveal the synergistic actions between organelles in living cells but also enable more in-depth dynamic monitoring of intracellular molecular networks;

(4) The design strategy for monitoring biomarkers: current research demands emphasize the design and development of fluorescent probes capable of single-molecule imaging (SIM) for biological markers by incorporating multiple recognition sites. These probes must exhibit high targeting efficiency and selectivity for accurate identification of specific biomarkers and high-resolution imaging. Additionally, the structural design of the probes should optimize their optical properties, stability, and biocompatibility to ensure effective performance in complex biological environments;

(5) The development of probes optimized for live-cell imaging and long-term tracking will enable continuous monitoring of organelles within living cells over extended periods. This is particularly valuable for real-time studies of dynamic processes such as cell signaling, organelle interactions, and disease progression;

(6) The future of fluorescent probes in SIM imaging lies in their potential applications for disease diagnosis and therapeutic monitoring. Probes targeting disease-specific biomarkers can be used to track organelle dysfunction associated with conditions such as cancer, neurodegenerative diseases, and metabolic disorders, enabling early detection and more personalized treatment strategies;

(7) Technological integration and expansion: combining SIM with other imaging modes such as photoactivation localization microscopy (PALM), stimulated emission loss microscopy (STED), and random optical reconstruction microscopy (STORM) can be used to construct a multimodal imaging platform, breaking through the limitation of SIM on axial resolution, and exploring its cross-scale research applications in

complex physiological/pathological processes. In addition, with the continuous advancement of computing power and image processing algorithms, it is possible to further optimize the image reconstruction process of SIM in the future, enhancing its potential for application in dynamic and large-scale samples.

Author contributions

All of the authors contributed to the manuscript preparation. Weiying Lin conceived the idea for this review and guided the project. Hua Zheng collected references; Guofang Li and Enxiang Ge participated in the image design; Guofang Li prepared the initial draft; Guofang Li, Enxiang Ge and Weiying Lin revised and edited the manuscript.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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References

1 S. D. Dyall, M. T. Brown and P. J. Johnson, Ancient invasions: from endosymbionts to organelles, *Science*, 2004, **304**, 253–257.
2 J. E. Lee, P. I. Cathey, H. Wu, R. Parker and A. G. K. Voeltz, Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles, *Science*, 2020, **367**, eaay7108.
3 G. Li, L. Zhang, H. Zheng, L. Huang, Z. Li, W. Li and W. Lin, Visualization of lysosomal dynamics during autophagy by fluorescent probe, *Anal. Chem.*, 2023, **95**, 15795–15802.
4 X. Liang, Y. Zhao, J. Yan, Q. Zhang, T. D. James and W. Lin, Mechanosensitive fluorescence lifetime probes for investigating the dynamic mechanism of ferroptosis, *Proc. Natl. Acad. Sci. U. S. A.*, 2024, **121**, e2316450121.
5 H. Zhu, J. Fan, J. Du and X. Peng, Fluorescent probes for sensing and imaging within specific cellular organelles, *Acc. Chem. Res.*, 2016, **49**, 2115–2126.
6 P. Gao, W. Pan, N. Li and B. Tang, Fluorescent probes for organelle-targeted bioactive species imaging, *Chem. Sci.*, 2019, **10**, 6035–6071.
7 Y. Zhao, H. S. Kim, X. Zou, L. Huang, X. Liang, J. S. Kim and W. Lin, Harnessing dual-fluorescence lifetime probes to validate regulatory mechanisms of organelle interactions, *J. Am. Chem. Soc.*, 2022, **144**, 20854–20865.
8 M. Zhu, W. Li, L. Sun, Z. Lv, X. Yang and Y. Wang, Advances in fluorescent probes for targeting organelles: Design strategies, applications and perspectives, *Coord. Chem. Rev.*, 2024, **512**, 215893.
9 Y. Wu and H. Shroff, Faster, sharper, and deeper: structured illumination microscopy for biological imaging, *Nat. Methods*, 2018, **15**, 1011–1019.
10 T. A. Klar, S. Jakobs, M. Dyba, A. Egner and S. W. Hell, Fluorescence microscopy with diffraction resolution barrier broken by stimulated emission, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 8206–8210.
11 A. Szymborska, A. De Marco, N. Daigle, V. C. Cordes, J. A. Briggs and J. Ellenberg, Nuclear pore scaffold structure analyzed by super-resolution microscopy and particle averaging, *Science*, 2013, **341**, 655–658.
12 M. J. Rust, M. Bates and X. Zhuang, Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM), *Nat. Methods*, 2006, **3**, 793–796.
13 R. Lin, E. T. Kipreos, J. Zhu, C. H. Khang and P. Kner, Subcellular three-dimensional imaging deep through multicellular thick samples by structured illumination microscopy and adaptive optics, *Nat. Commun.*, 2021, **12**, 3148.
14 X. Chen, S. Zhong, Y. Hou, R. Cao, W. Wang, D. Li, Q. Dai, D. Kim and P. Xi, Superresolution structured illumination microscopy reconstruction algorithms: a review, *Light: Sci. Appl.*, 2023, **12**, 172.
15 X. Xu, K. Qiu, Z. Tian, C. Aryal, F. Rowan, R. Chen, Y. Sun and J. Diao, Probing the dynamic crosstalk of lysosomes and mitochondria with structured illumination microscopy, *Trends Anal. Chem.*, 2023, **169**, 117370.
16 K. N. Wang, X. Shao, Z. Tian, L. Y. Liu, C. Zhang, C. P. Tan, J. Zhang, P. Ling, F. Liu, Q. Chen, J. Diao and Z. W. Mao, A continuous add-on probe reveals the nonlinear enlargement of mitochondria in light-activated oncosis, *Adv. Sci.*, 2021, **8**, 2004566.
17 Pragti, B. K. Kundu, R. Chen, J. Diao and Y. Sun, Near-Infrared Bioimaging Using Two-photon Fluorescent Probes, *Adv. Healthcare Mater.*, 2025, **14**, 2403272.
18 H. Fang, Y. Chen, S. Geng, S. Yao, Z. Guo and W. He, Super-resolution imaging of mitochondrial HClO during cell ferroptosis using a near-infrared fluorescent probe, *Anal. Chem.*, 2022, **94**, 17904–17912.
19 C. Zhang, H. Shao, J. Zhang, X. Guo, Y. Liu, Z. Song, F. Liu, P. Ling, L. Tang, K. N. Wang and Q. Chen, Long-term live-cell lipid droplet-targeted biosensor development for nanoscopic tracking of lipid droplet-mitochondria contact sites, *Theranostics*, 2021, **11**, 7767–7778.
20 L. Y. Liu, H. Fang, Q. Chen, M. H. Y. Chan, M. Ng, K. N. Wang, W. Liu, Z. Tian, J. Diao, Z. W. Mao and V. W. W. Yam, Multiple-Color Platinum Complex with Super-Large Stokes Shift for Super-Resolution Imaging of

Autolysosome Escape, *Angew. Chem., Int. Ed.*, 2020, **132**, 19391–19398.

21 C. Du, S. Fu, X. Wang, A. C. Sedgwick, W. Zhen, M. Li, X. Li, J. Zhou, Z. Wang, H. Wang and J. L. Sessler, Diketopyrrolopyrrole-based fluorescence probes for the imaging of lysosomal Zn^{2+} and identification of prostate cancer in human tissue, *Chem. Sci.*, 2019, **10**, 5699–5704.

22 B. Guo, J. Jing, L. Nie, F. Xin, C. Gao, W. Yang and X. Zhang, A lysosome targetable versatile fluorescent probe for imaging viscosity and peroxynitrite with different fluorescence signals in living cells, *J. Mater. Chem. B*, 2018, **6**, 580–585.

23 F. Ali, S. Aute, S. Sreedharan, H. A. Anila, H. K. Saeed, C. G. Smythe, J. A. Thomas and A. Das, Tracking HOCl concentrations across cellular organelles in real time using a super resolution microscopy probe, *Chem. Commun.*, 2018, **54**, 1849–1852.

24 X. C. Chen, G. X. Tang, W. H. Luo, W. Shao, J. Dai, S. T. Zeng, Z. S. Huang, S. B. Chen and J. H. Tan, Monitoring and modulating mtDNA G-quadruplex dynamics reveal its close relationship to cell glycolysis, *J. Am. Chem. Soc.*, 2021, **143**, 20779–20791.

25 Y. Tang, X. Kong, Z. R. Liu, A. Xu and W. Lin, Lysosome-targeted turn-on fluorescent probe for endogenous formaldehyde in living cells, *Anal. Chem.*, 2016, **88**, 9359–9363.

26 G. Lukinavicius, C. Blaukopf, E. Pershagen, A. Schena, L. Reymond, E. Derivery, M. Gonzalez-Gaitan, E. D'Este, S. W. Hell, D. Wolfram Gerlich and K. Johnsson, SiR–Hoechst is a far-red DNA stain for live-cell nanoscopy, *Nat. Commun.*, 2015, **6**, 8497.

27 S. Jakobs, T. Stephan, P. Ilgen and C. Brüser, Light microscopy of mitochondria at the nanoscale, *Annu. Rev. Biophys.*, 2020, **49**, 289–308.

28 M. Saxena, G. Eluru and S. S. Gorthi, Structured illumination microscopy, *Adv. Opt. Photonics*, 2015, **7**, 241–275.

29 X. Xu, K. Qiu, Z. Tian, C. Aryal, F. Rowan, R. Chen, Y. Sun and J. Diao, Probing the dynamic crosstalk of lysosomes and mitochondria with structured illumination microscopy, *Trends Anal. Chem.*, 2023, **169**, 117370.

30 R. Heintzmann and T. Huser, Super-resolution structured illumination microscopy, *Chem. Rev.*, 2017, **117**, 13890–13908.

31 X. Huang, J. Fan, L. Li, H. Liu, R. Wu, Y. Wu, L. Wei, H. Mao, A. Lal, P. Xi, L. Tang, Y. Zhang, Y. Liu, S. Tan and L. Chen, Fast, long-term, super-resolution imaging with Hessian structured illumination microscopy, *Nat. Biotechnol.*, 2018, **36**, 451–459.

32 M. Tian, J. Zhan and W. Lin, Single fluorescent probes enabling simultaneous visualization of duple organelles: Design principles, mechanisms, and applications, *Coord. Chem. Rev.*, 2022, **451**, 214266.

33 J. Yin, L. Huang, L. Wu, J. Li, T. D. James and W. Lin, Small molecule based fluorescent chemosensors for imaging the microenvironment within specific cellular regions, *Chem. Soc. Rev.*, 2021, **50**, 12098–12150.

34 R. Chen, K. Qiu, D. C. Y. Leong, B. K. Kundu, C. Zhang, P. Srivastava, K. E. White, G. Li, G. Han, Z. Guo, C. G. Elles, J. Diao and Y. Sun, A general design of pyridinium-based fluorescent probes for enhancing two-photon microscopy, *Biosens. Bioelectron.*, 2023, **239**, 115604.

35 L. Wang, M. S. Frei, A. Salim and K. Johnsson, Small-molecule fluorescent probes for live-cell super-resolution microscopy, *J. Am. Chem. Soc.*, 2019, **141**, 2770–2781.

36 X. Shao, C. Meng, W. Song, T. Zhang and Q. Chen, Subcellular visualization: organelle-specific targeted drug delivery and discovery, *Adv. Drug Delivery Rev.*, 2023, **199**, 114977.

37 W. Zou, L. Yang, H. Lu, M. Li, D. Ji, J. Slone and T. Huang, Application of super-resolution microscopy in mitochondria-dynamic diseases, *Adv. Drug Delivery Rev.*, 2023, **200**, 115043.

38 C. Zhang, Z. Tian, R. Chen, F. Rowan, K. Qiu, Y. Sun, J. L. Guan and J. Diao, Advanced imaging techniques for tracking drug dynamics at the subcellular level, *Adv. Drug Delivery Rev.*, 2023, **201**, 114978.

39 J. R. Friedman and J. Nunnari, Mitochondrial form and function, *Nature*, 2014, **505**, 335–343.

40 S. Delaunay, G. Pascual, B. Feng, K. Klann, M. Behm, A. Hotz-Wagenblatt, K. Richter, K. Zaoui, E. Herpel, C. Munch, S. Dietmann, J. Hess, S. A. Benitah and M. Frye, Mitochondrial RNA modifications shape metabolic plasticity in metastasis, *Nature*, 2022, **607**, 593–603.

41 J. S. Harrington, S. W. Ryter, M. Plataki, D. R. Price and A. M. K. Choi, Mitochondria in health, disease, and aging, *Physiol. Rev.*, 2023, **103**, 2349–2422.

42 G. S. Gorman, P. F. Chinnery, S. DiMauro, M. Hirano, Y. Koga, R. McFarland, A. Suomalainen, D. R. Thorburn, M. Zeviani and D. M. Turnbull, Mitochondrial diseases, *Nat. Rev. Dis. Primers*, 2016, **2**, 16080.

43 J. Chow, J. Rahman, J. Achermann, M. T. Dattani and S. Rahman, Mitochondrial disease and endocrine dysfunction, *Nat. Rev. Endocrinol.*, 2017, **13**, 92–104.

44 T. B. Ren, Q. L. Zhang, D. Su, X. X. Zhang, L. Yuan and X. B. Zhang, Detection of analytes in mitochondria without interference from other sites based on an innovative ratiometric fluorophore, *Chem. Sci.*, 2018, **9**, 5461–5466.

45 L. Wang, Y. Ma, Z. Li, S. Li and W. Lin, A multifunctional fluorescent probe for dual-color visualization of intracellular mitochondria and lipid droplets and monitoring of SO_2 in vivo, *Chem. Eng. J.*, 2023, **451**, 139023.

46 G. Cheng, J. Fan, W. Sun, K. Sui, X. Jin, J. Wang and X. Peng, A highly specific BODIPY-based probe localized in mitochondria for HClO imaging, *Analyst*, 2013, **138**, 6091–6096.

47 X. Li, Y. Zhao, J. Yin and W. Lin, Organic fluorescent probes for detecting mitochondrial membrane potential, *Coord. Chem. Rev.*, 2020, **420**, 213419.

48 W. Meng, R. Chen, Q. Jang, K. Ma, D. Li, H. Xiao, W. Chi, C. Zeng and W. Sun, Recent mitochondria-immobilized

fluorescent probes for high-fidelity bioimaging: From design to application, *Coord. Chem. Rev.*, 2025, **529**, 216456.
49 R. Zhai, B. Fang, Y. Lai, B. Peng, H. Bai, X. Liu, L. Li and W. Huang, Small-molecule fluorogenic probes for mitochondrial nanoscale imaging, *Chem. Soc. Rev.*, 2023, **52**, 942–972.
50 X. Gu, E. Zhao, T. Zhao, M. Kang, C. Gui, J. W. Y. Lam, S. Du, M. M. T. Loy and B. Z. Tang, A mitochondrion-specific photoactivatable fluorescence turn-on AIE-based bioprobe for localization super-resolution microscope, *Adv. Mater.*, 2016, **28**, 5064–5071.
51 J. Yin, X. Zheng, Y. Zhao, X. Shen, T. Cheng, X. Shao, X. Jing, S. Huang and W. Lin, Investigating the Therapeutic Effects of Ferroptosis on Myocardial Ischemia-Reperfusion Injury Using a Dual-Locking Mitochondrial Targeting Strategy, *Angew. Chem., Int. Ed.*, 2024, **63**, e202402537.
52 C. P. Satori, M. M. Henderson, E. A. Krautkramer, V. Kostal, M. D. Distefano and E. A. Arriaga, Bioanalysis of eukaryotic organelles, *Chem. Rev.*, 2013, **113**, 2733–2811.
53 G. Jiang, H. Liu, H. Liu, G. Ke, T. B. Ren, B. Xiong, X. B. Zhang and L. Yuan, Chemical approaches to optimize the properties of organic fluorophores for imaging and sensing, *Angew. Chem., Int. Ed.*, 2024, e202315217.
54 Y. Liu, J. Zhou, L. Wang, X. Hu, X. Liu, M. Liu, Z. Cao, D. Shangguan and W. Tan, A cyanine dye to probe mitophagy: simultaneous detection of mitochondria and autolysosomes in live cells, *J. Am. Chem. Soc.*, 2016, **138**, 12368–12374.
55 W. Liu, Q. Qiao, J. Zheng, J. Chen, W. Zhou, N. Xu, J. Li, L. Miao and Z. Xu, An assembly-regulated SNAP-tag fluorogenic probe for long-term super-resolution imaging of mitochondrial dynamics, *Biosens. Bioelectron.*, 2021, **176**, 112886.
56 Y. Zhu, R. Jia, Z. Ke, X. Wang, L. Su, Y. Tian and X. Tian, Carbazole-based fluorescent probe with a Large Stokes Shift for three-dimensional dynamic ultra-structures to monitor mitochondria in living cells, *Dyes Pigm.*, 2023, **208**, 110875.
57 Z. Wu, C. Zhang, J. Sha, Z. Jing, J. He, Y. Bai, J. Wu, S. Zhang and P. Shi, Ultrabright Xanthene Fluorescence Probe for Mitochondrial Super-Resolution Imaging, *Anal. Chem.*, 2024, **96**, 5134–5142.
58 G. Li, H. Zheng, L. Zhang, L. Huang and W. Lin, Mitochondria-Specific Fluorescent Probe for Revealing the Interaction between Mitochondria and Lysosomes during Apoptosis, *Anal. Chem.*, 2024, **96**, 14291–14297.
59 Y. Ren, L. L. Wu, W. Song, Y. Gao, L. Shao, Z. Lu, S. Wang, X. Shao, Z. Yu, M. Zhang, J. Eu, L. Han, K. Zeng and Q. Chen, Mitochondrial Electron Flow Dynamics Imaging for Assessing Mitochondrial Quality and Drug Screening, *Adv. Sci.*, 2025, 2410561.
60 Q. Qiao, W. Liu, W. Chi, J. Chen, W. Zhou, N. Xu, J. Li, X. Fang, Y. Tao, Y. Zhang, Y. Chen, L. Miao, X. Liu and Z. Xu, Modulation of dynamic aggregation in fluorogenic SNAP-tag probes for long-term super-resolution imaging, *Aggregate*, 2022, **4**, e258.
61 N. Xu, Q. Qiao, Y. Tao, P. Bao, Y. Zhang, W. Zhou, J. Li, Z. Li and Z. Xu, Protein proximity ligation probe with wide sensing range resolving dynamics of subcellular ph by super-resolution imaging, *Sens. Actuators, B*, 2024, **398**, 134744.
62 K. N. Wang, X. Shao, Z. Tian, L. Y. Liu, C. Zhang, C. P. Tan, J. Zhang, P. Ling, F. Liu, Q. Chen, J. Diao and Z. W. Mao, A continuous add-on probe reveals the nonlinear enlargement of mitochondria in light-activated oncosis, *Adv. Sci.*, 2021, **8**, 2004566.
63 Q. Chen, L. Y. Liu, Z. Tian, Z. Fang, K. N. Wang, X. Shao, C. Zhang, W. Zou, F. Rowan, K. Qiu, B. Ji, J. L. Guan, D. Li, Z. W. Mao and J. Diao, Mitochondrial nucleoid condensates drive peripheral fission through high membrane curvature, *Cell Rep.*, 2023, **42**, 113472.
64 B. Liu, T. Sun, Y. Wang, X. Y. Xia, S. Cao, K. N. Wang, Q. Chen and Z. W. Mao, Real-time monitoring of mtDNA aggregation and mitophagy induced by a fluorescent platinum complex in living cells, *Anal. Chem.*, 2024, **96**, 13421–13428.
65 X. C. Chen, G. X. Tang, W. H. Luo, W. Shao, J. Dai, S. T. Zeng, Z. S. Huang, S. B. Chen and J. H. Tan, Monitoring and modulating mtDNA G-quadruplex dynamics reveal its close relationship to cell glycolysis, *J. Am. Chem. Soc.*, 2021, **143**, 20779–20791.
66 Q. Ding, X. Wang, Y. Luo, X. Leng, X. Li, M. Gu and J. S. Kim, Mitochondria-targeted fluorophore: State of the art and future trends, *Coord. Chem. Rev.*, 2024, **508**, 215772.
67 A. S. Manolis, A. A. Manolis, T. A. Manolis, N. E. Apostolaki, E. J. Apostolopoulos, H. Melita and N. Katsiki, Mitochondrial dysfunction in cardiovascular disease: Current status of translational research/clinical and therapeutic implications, *Med. Res. Rev.*, 2021, **41**, 275–313.
68 J. Nunnari and A. Suomalainen, Mitochondria: in sickness and in health, *Cell*, 2012, **148**, 1145–1159.
69 Y. Geng, Z. Wang, J. Zhou, M. Zhu, J. Liu and T. D. James, Recent progress in the development of fluorescent probes for imaging pathological oxidative stress, *Chem. Soc. Rev.*, 2023, **52**, 3873–3926.
70 W. Y. Lu, H. J. Li and Y. C. Wu, Assessment of environmental and biological stress using mitochondria-targeted red-emitting and near-infrared fluorescent probes for biothiol analysis: A review, *Environ. Chem. Lett.*, 2024, **22**, 3135–3169.
71 Y. Liu, C. Zhang, Y. Wei, H. Chen, L. Kong, Q. Chen and Y. Wang, De Novo-Designed landmine warfare strategy luminophore for super-resolution imaging reveal $ONOO^-$ evolution in living cells, *Chem. Eng. J.*, 2021, **422**, 130151.
72 Y. Wei, L. Kong, H. Chen, Y. Liu, Y. Xu, H. Wang, G. Fang, X. Shao, F. Liu, Y. Wang and Q. Chen, Super-resolution image-based tracking of drug distribution in mitochondria of a label-free naturally derived drug molecules, *Chem. Eng. J.*, 2022, **429**, 132134.
73 M. Hu, B. Wang, H. Zhang, H. Wang, H. Li, X. Zhang, J. Zhang, Q. Lu, G. Fang, J. Wang and B. Dong, A dual-labeling probe for super-resolution imaging to detect

mitochondrial reactive sulfur species in live cells, *Front. Pharmacol.*, 2022, **13**, 871059.

74 H. Fang, Y. Chen, S. Geng, S. Yao, Z. Guo and W. He, Super-resolution imaging of mitochondrial HClO during cell ferroptosis using a near-infrared fluorescent probe, *Anal. Chem.*, 2022, **94**, 17904–17912.

75 H. Chen, G. Fang, Y. Ren, W. Zou, K. Ying, Z. Yang and Q. Chen, Super-resolution imaging for in situ monitoring sub-cellular micro-dynamics of small molecule drug, *Acta Pharm. Sin. B*, 2024, **14**, 1864–1877.

76 B. Fang, L. Wang, H. Li, J. Zhang, Y. Ding, P. Li, B. Peng, H. Bai and L. Li, Deciphering the role of mtH_2O_2 in hepatic ischemia-reperfusion injury mechanisms with low-background super-resolution fluorogenic probe, *Sci. China: Chem.*, 2025, **68**, 297–307.

77 M. Holtmannspötter, E. Wienbeuker, T. Dellmann, I. Watrinet, A. J. Garcia-Sáez, K. Johnsson, R. Kurre and J. Piehler, Reversible Live-Cell Labeling with Retro-engineered HaloTags Enables Long-Term High-and Super-Resolution Imaging, *Angew. Chem., Int. Ed.*, 2023, **62**, e202219050.

78 R. Huang, C. H. Huang, J. Chen, Z. Y. Yan, M. Tang, J. Shao, K. Cai and B. Z. Zhu, Unprecedented enantio-selective live-cell mitochondrial DNA super-resolution imaging and photo-sensitizing by the chiral ruthenium polypyridyl DNA "light-switch", *Nucleic Acids Res.*, 2023, **51**, 11981–11998.

79 S. Zheng, N. Dadina, D. Mozumdar, L. Lesiak, K. N. Martinez, E. W. Miller and A. Schepartz, Long-term super-resolution inner mitochondrial membrane imaging with a lipid probe, *Nat. Chem. Biol.*, 2024, **20**, 83–92.

80 A. Zadoorian, X. Du and H. Yang, Lipid droplet biogenesis and functions in health and disease, *Nat. Rev. Endocrinol.*, 2023, **19**, 443–459.

81 W. Zhu, X. Zheng, Y. Huang, Z. Lu and H. Ai, Super-resolution imaging and real-time tracking lysosome in living cells by a fluorescent probe, *Sci. China: Chem.*, 2018, **61**, 483–489.

82 X. Zheng, W. Zhu, F. Ni, H. Ai and C. Yang, A specific bioprobe for super-resolution fluorescence imaging of lipid droplets, *Sens. Actuators, B*, 2018, **255**, 3148–3154.

83 Q. Shen, R. Xu, Z. Wang, T. Zhao, Y. Zhou, Y. Xu, Z. Yang, M. Lei, L. Meng and D. Dang, Donor-Acceptor Typed AIE Luminogens with Near-infrared Emission for Super-resolution Imaging, *Chem. Res. Chin. Univ.*, 2021, **37**, 143–149.

84 M. Y. Wu, J. K. Leung, C. Kam, T. Chou, D. Wang, S. Feng and S. Chen, A near-infrared AIE probe for super-resolution imaging and nuclear lipid droplet dynamic study, *Mater. Chem. Front.*, 2021, **5**, 3043–3049.

85 C. Zhang, H. Shao, J. Zhang, X. Guo, Y. Liu, Z. Song, F. Liu, P. Ling, L. Tang, K. N. Wang and Q. Chen, Long-term live-cell lipid droplet-targeted biosensor development for nanoscopic tracking of lipid droplet-mitochondria contact sites, *Theranostics*, 2021, **11**, 7767–7778.

86 J. Chen, C. Wang, W. Liu, Q. Qiao, H. Qi, W. Zhou, N. Xu, J. Li, H. Piao, D. Tan, X. Liu and Z. Xu, Stable super-resolution imaging of lipid droplet dynamics through a buffer strategy with a hydrogen-bond sensitive fluorogenic probe, *Angew. Chem., Int. Ed.*, 2021, **133**, 25308–25317.

87 M. Cao, T. Zhu, M. Zhao, F. Meng, Z. Liu, J. Wang, G. Niu and X. Yu, Structure rigidification promoted ultrabright solvatochromic fluorescent probes for super-resolution imaging of cytosolic and nuclear lipid droplets, *Anal. Chem.*, 2022, **94**, 10676–10684.

88 J. Chen, W. Liu, X. Fang, Q. Qiao and Z. Xu, BODIPY 493 acts as a bright buffering fluorogenic probe for super-resolution imaging of lipid droplet dynamics, *Chin. Chem. Lett.*, 2022, **33**, 5042–5046.

89 J. Dai, Z. Wu, D. Li, G. Peng, G. Liu, R. Zhou, C. Wang, X. Yan, F. Liu, P. Sun, J. Zhou and G. Lu, Super-resolution dynamic tracking of cellular lipid droplets employing with a photostable deep red fluorogenic probe, *Biosens. Bioelectron.*, 2023, **229**, 115243.

90 Z. Huo, Z. Yu, W. Xu and S. Xu, Super-Resolution Microscopic Imaging of Lipid Droplets in Living Cells via Carbonized Polymer Dot-Based Polarity-Responsive Nanoprobe, *ACS Meas. Sci. Au*, 2024, **4**, 593–598.

91 N. Xu, Q. Qiao, X. Fang, G. Wang, K. An, W. Jiang, J. Li and Z. Xu, Solvatochromic buffering fluorescent probe resolves the lipid transport and morphological changes during lipid droplet fusion by super-resolution imaging, *Anal. Chem.*, 2024, **96**, 4709–4715.

92 J. Chen, Q. Qiao, H. Wang, W. Jiang, W. Liu, K. An and Z. Xu, Clog P-Guided Development of Multi-Colored Buffering Fluorescent Probes for Super-Resolution Imaging of Lipid Droplet Dynamics, *Adv. Sci.*, 2024, **11**, 2408030.

93 Y. Wang, Y. Song, L. Xu, W. Zhou, W. Wang, Q. Jin, Y. Xie, J. Zhang, J. Liu, W. Wu, H. Li, L. Liang, J. Wang, Y. Yang, X. Chen, S. Ge, T. Gao, L. Zhang and M. Xie, A Membrane-Targeting Aggregation-Induced Emission Probe for Monitoring Lipid Droplet Dynamics in Ischemia/Reperfusion-Induced Cardiomyocyte Ferroptosis, *Adv. Sci.*, 2024, **11**, 2309907.

94 M. Liu, W. Peng, H. Zheng, K. Chen, Q. Lin, S. Zhang and L. Yang, Assessing atherosclerosis by super-resolution imaging of HClO in foam cells using a ratiometric fluorescent probe, *Anal. Chem.*, 2024, **96**, 14215–14221.

95 L. Kong, Q. Bai, C. Li, Q. Wang, Y. Wang, X. Shao, Y. Wei, J. Sun, Z. Yu, J. Yin, B. Shi, H. Fang, X. Chen and Q. Chen, Molecular probes for tracking lipid droplet membrane dynamics, *Nat. Commun.*, 2024, **15**, 9413.

96 J. P. Luzio, P. R. Pryor and N. A. Bright, *Nat. Rev. Mol. Cell Biol.*, 2007, **8**, 622–632.

97 F. M. Platt, Emptying the stores: lysosomal diseases and therapeutic strategies, *Nat. Rev. Drug Discovery*, 2018, **17**, 133–150.

98 Z. Lv, Z. Man, H. Cui, Z. Xu, H. Cao, S. Li, Q. Liao, Q. He, L. Zheng and B. Fu, Red AIE luminogens with tunable organelle specific anchoring for live cell dynamic super resolution imaging, *Adv. Funct. Mater.*, 2020, **31**, 2009329.

99 Z. Ye, W. Yang, C. Wang, Y. Zheng, W. Chi, X. Liu, Z. Huang, X. Li and Y. Xiao, Quaternary piperazine-substituted rhodamines with enhanced brightness for super-resolution imaging, *J. Am. Chem. Soc.*, 2019, **141**, 14491–14495.

100 Q. Qiao, W. Yin, X. Wu, S. Wu, Y. Ruan, N. Xu, J. Li, Z. S. Wu, X. Liu and Z. Xu, Two-Color Single-Molecule Blinking Ratiometricity: A Functional Super-Resolution Imaging Approach for Resolving Lysosomal pH and Dynamics, *Angew. Chem., Int. Ed.*, 2025, e202503916.

101 K. Qiu, A. Yadav, Z. Tian, Z. Guo, D. Shi, C. K. Nandi and J. Diao, Ultralong-term super-resolution tracking of lysosomes in brain organoids by near-infrared noble metal nanoclusters, *ACS Mater. Lett.*, 2022, **4**, 1565–1573.

102 T. Sun, H. Wang, X. Zhang, P. Ling, Y. Xiao, Q. Chen and X. Chen, Ion monitoring at nanoscale sites of interorganelle membrane contact in living cells, *Small Struct.*, 2024, **5**, 2300350.

103 D. Zhao, J. Wang, L. Gao, X. Huang, F. Zhu and F. Wang, Visualizing the intracellular aggregation behavior of gold nanoclusters via structured illumination microscopy and scanning transmission electron microscopy, *Sci. Total Environ.*, 2024, **912**, 169153.

104 Z. Yu, H. Shao, X. Shao, L. Yu, Y. Gao, Y. Ren, F. Liu, C. Meng, P. Ling and Q. Chen, In situ visualization of the cellular uptake and sub-cellular distribution of mussel oligosaccharides, *J. Pharm. Anal.*, 2024, **14**, 100932.

105 H. Zheng, W. Peng, M. Liu, S. Zhang, X. Song and L. Yang, Ratiometric fluorescent probe for super-resolution imaging of lysosome HClO in ferroptosis cells, *Anal. Chem.*, 2024, **96**, 11581–11587.

106 D. E. Clapham, Calcium signaling, *Cell*, 2007, **131**, 1047–1058.

107 G. van Meer, D. R. Voelker and G. W. Feigenson, Membrane lipids: where they are and how they behave, *Nat. Rev. Mol. Cell Biol.*, 2008, **9**, 112–124.

108 M. Okumura, K. Noi, S. Kanemura, M. Kinoshita, T. Saio, Y. Inoue, T. Hikima, S. Akiyama, T. Ogura and K. Inaba, Dynamic assembly of protein disulfide isomerase in catalysis of oxidative folding, *Nat. Chem. Biol.*, 2019, **15**, 499–509.

109 L. Guo, M. Tian, Z. Zhang, Q. Lu, Z. Liu, G. Niu and X. Yu, Simultaneous two-color visualization of lipid droplets and endoplasmic reticulum and their interplay by single fluorescent probes in lambda mode, *J. Am. Chem. Soc.*, 2021, **143**, 3169–3179.

110 Z. Zhu, Q. Wang, H. Liao, M. Liu, Z. Liu, Y. Zhang and W. Zhu, Trapping endoplasmic reticulum with amphiphilic AIE-active sensor via specific interaction of ATP-sensitive potassium (KATP), *Natl. Sci. Rev.*, 2021, **8**, nwaa198.

111 A. Fujisawa, T. Tamura, Y. Yasueda, K. Kuwata and I. Hamachi, Chemical profiling of the endoplasmic reticulum proteome using designer labeling reagents, *J. Am. Chem. Soc.*, 2018, **140**, 17060–17070.

112 H. Fang, L. Hu, Q. Chen, S. Geng, K. Qiu, C. Wang, M. Hao, Z. Tian, H. Chen, L. Liu, J. L. Guan, Y. Chen, L. Dong, Z. Guo, W. He and J. Diao, An ER-targeted "reserve-release" fluorogen for topological quantification of reticulophagy, *Biomaterials*, 2023, **292**, 121929.

113 K. Han, S. Huang, J. Kong, Y. Yang, L. Shi and Y. Ci, A novel fluorescent endoplasmic reticulum marker for super-resolution imaging in live cells, *FEBS Lett.*, 2023, **597**, 693–701.

114 P. Xu, S. Zhong, Y. Wei, X. Duan, M. Zhang, W. Shen, Y. Ma and Y. H. Zhang, Surface-functionalized halo-tag gold nanoprobes for live-cell long-term super-resolution imaging of endoplasmic reticulum dynamics, *ACS Nano*, 2024, **18**, 21433–21446.

115 K. Jacobson, O. G. Mouritsen and R. G. W. Anderson, Lipid rafts: at a crossroad between cell biology and physics, *Nat. Cell Biol.*, 2007, **9**, 7–14.

116 T. A. Sanders, E. Llagostera and M. Barna, Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning, *Nature*, 2013, **497**, 628–632.

117 H. Pick, A. C. Alves and H. Vogel, Single-vesicle assays using liposomes and cell-derived vesicles: from modeling complex membrane processes to synthetic biology and biomedical applications, *Chem. Rev.*, 2018, **118**, 8598–8654.

118 M. Mathieu, L. Martin-Jaular, G. Lavieu and C. Théry, Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication, *Nat. Cell Biol.*, 2019, **21**, 9–17.

119 B. L. Heckmann, B. J. W. Teubner, B. Tummers, E. Boada-Romero, L. Harris, M. Yang, C. S. Guy, S. S. Zakharenko and D. R. Green, LC3-associated endocytosis facilitates β-amyloid clearance and mitigates neurodegeneration in murine Alzheimer's disease, *Cell*, 2019, **178**, 536–551.

120 J. Garcia-Calvo, J. Maillard, I. Fureraj, K. Strakova, A. Colom, V. Mercier, A. Roux, E. Vauthey, N. Sakai, A. Fürstenberg and S. Matile, Fluorescent membrane tension probes for super-resolution microscopy: Combining mechanosensitive cascade switching with dynamic-covalent ketone chemistry, *J. Am. Chem. Soc.*, 2020, **142**, 12034–12038.

121 K. Qiu, R. Seino, G. Han, M. Ishiyama, Y. Ueno, Z. Tian, Y. Sun and J. Diao, De Novo Design of A Membrane-Anchored Probe for Multidimensional Quantification of Endocytic Dynamics, *Adv. Healthcare Mater.*, 2022, **11**, 2102185.

122 L. Miao, C. Yan, Y. Chen, W. Zhou, X. Zhou, Q. Qiao and Z. Xu, SIM imaging resolves endocytosis of SARS-CoV-2 spike RBD in living cells, *Cell Chem. Biol.*, 2023, **30**, 248–260.

123 K. An, Q. Qiao, W. Zhou, W. Jiang, J. Li and Z. Xu, Stable Super-Resolution Imaging of Cell Membrane Nanoscale Subcompartment Dynamics with a Buffering Cyanine Dye, *Anal. Chem.*, 2024, **96**, 5985–5991.

124 W. Zhou, Y. Tao, Q. Qiao, N. Xu, J. Li, G. Wang, X. Fang, J. Chen, W. Liu and Z. Xu, Cell-Impermeable Buffering Fluorogenic Probes for Live-Cell Super-Resolution Imaging of Plasma Membrane Morphology Dynamics, *ACS Sens.*, 2024, **9**, 3170–3177.

125 M. J. Phillips and G. K. Voeltz, Structure and function of ER membrane contact sites with other organelles, *Nat. Rev. Mol. Cell Biol.*, 2016, **17**, 69–82.
126 R. Filadi, E. Greotti, G. Turacchio, A. Luini, T. Pozzan and P. Pizzo, Mitofusin 2 ablation increases endoplasmic reticulum–mitochondria coupling, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, **112**, 2174–2181.
127 Y. C. Wong, S. Kim, W. Peng and D. Krainc, Regulation and function of mitochondria–lysosome membrane contact sites in cellular homeostasis, *Trends Cell Biol.*, 2019, **29**, 500–513.
128 Q. Chen, H. Fang, X. Shao, Z. Tian, S. Geng, Y. Zhang, H. Fan, P. Xiang, J. Zhang, X. Tian, K. Zhang, W. He, Z. Guo and J. Diao, A dual-labeling probe to track functional mitochondria–lysosome interactions in live cells, *Nat. Commun.*, 2020, **11**, 6290.
129 Q. Chen, C. Jin, X. Shao, R. Guan, Z. Tian, C. Wang, F. Liu, P. Ling, J. L. Guan, L. Ji, F. Wang, H. Chao and J. Diao, Super-resolution tracking of mitochondrial dynamics with an Iridium (III) luminophore, *Small*, 2018, **14**, 1802166.
130 Q. Chen, X. Shao, M. Hao, H. Fang, R. Guan, Z. Tian, M. Li, C. Wang, L. Ji, H. Chao, J. L. Guan and J. Diao, Quantitative analysis of interactive behavior of mitochondria and lysosomes using structured illumination microscopy, *Biomaterials*, 2020, **250**, 120059.
131 H. Fang, S. Geng, M. Hao, Q. Chen, M. Liu, Z. Tian, C. Wang, T. Takebe, J. L. Guan, Y. Chen, Z. Guo, W. He and J. Diao, Simultaneous Zn^{2+} tracking in multiple organelles using super-resolution morphology-correlated organelle identification in living cells, *Nat. Commun.*, 2021, **12**, 109.
132 H. Chen, H. Wang, Y. Wei, M. Hu, B. Dong, H. Fang and Q. Chen, Super-resolution imaging reveals the subcellular distribution of dextran at the nanoscale in living cells, *Chin. Chem. Lett.*, 2022, **33**, 1865–1869.
133 G. Fang, H. Chen, X. Shao, H. Wang, D. Zhan, R. Wang, P. Meng, H. Fang, F. Liu, P. Ling, Z. Wu, J. Diao, Q. Yao and Q. Chen, Single image capture of bioactive ion crosstalk within inter-organelle membrane contacts at nanometer resolution, *Small Methods*, 2022, **6**, 2200321.
134 G. Byun, S. Y. Kim, M. W. Choi, J. K. Yang, J. Kwon, S. Kim and S. Park, Highly photostable fluorescent probes for multi-color and super-resolution imaging of cell organelles, *Dyes Pigm.*, 2022, **204**, 110427.
135 M. Tian, Y. Ma and W. Lin, Fluorescent probes for the visualization of cell viability, *Acc. Chem. Res.*, 2019, **52**, 2147–2157.
136 R. Chen, K. Qiu, G. Han, B. K. Kundu, G. Ding, Y. Sun and J. Diao, Quantifying cell viability through organelle ratiometric probing, *Chem. Sci.*, 2023, **14**, 10236–10248.
137 D. Liu, G. Fang, Y. Wang, C. Meng, Z. Liu, Q. Chen and X. Shao, Facile construction of dual-response super-resolution probes for tracking organelles dynamics, *Exploration*, 2024, **4**, 20230145.
138 P. Xu, S. Zhong, Y. Ma, X. Duan, Y. Wei, M. Zhang, J. Xu and Y. H. Zhang, Delivery of Fluorescent Nanoprobes into the Cytoplasm Using Cell-Penetrating Peptides for Live-Cell Super-Resolution Fluorescence Imaging, *ACS Appl. Nano Mater.*, 2024, **7**, 2120–2131.
139 Y. Lai, T. Zhang, L. Huang, A. S. Klymchenko and W. Lin, An exchangeable SIM probe for monitoring organellar dynamics of necrosis cells and intracellular water heterogeneity in kidney repair, *Proc. Natl. Acad. Sci. U. S. A.*, 2025, **122**, e2402348121.