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Organelle-Targeted AIE Probes for Cell Sorting

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Synonyms

AIE-based cell sorting tools; Cell sorting using organelle-targeting fluorescent probes; Organelle-specific AIE probes for cell fractionation; Subcellular-localized AIE probes for cell separation

Definition

Organelle-targeted probes are a specialized class of fluorescent probes designed to specifically target and visualize particular organelles within living cells.

Cell nucleus is a membrane-bound organelle found in eukaryotic cells. It houses the cell's genetic material, deoxyribonucleic acid (DNA), which is organized into chromosomes. The nucleus is essential for controlling gene expression, regulating cellular activities, and transmitting genetic information to daughter cells during cell division.

Cell membrane, also known as the plasma membrane, is a biological barrier that separates the intracellular environment from the extracellular environment. It is a phospholipid bilayer composed of two layers of phospholipids arranged tail-to-tail. The hydrophilic heads of the phospholipids face the aqueous environment on both sides of the membrane, while the hydrophobic tails face each other in the interior of the membrane.

Mitochondria are membrane-bound organelles found in most eukaryotic cells. Often referred to as the "powerhouses of the cell," they are responsible for generating adenosine triphosphate (ATP), the primary energy currency used by cells. Mitochondria are essential for cellular respiration, a metabolic process that converts nutrients into ATP.

Lysosomes are membrane-bound organelles found in eukaryotic cells that are responsible for the degradation of cellular waste products and foreign substances. They contain a variety of hydrolytic enzymes, including proteases, lipases, nucleases, and glycosidases, that can break down a wide range of molecules.

Lipid droplets (LDs) are intracellular organelles that store neutral lipids, such as triglycerides and sterol esters. They are spherical structures surrounded by a single phospholipid monolayer. Lipid droplets are found in a variety of cell types, including adipocytes, hepatocytes, and muscle cells.

The endoplasmic reticulum (ER) is a membranous organelle found in eukaryotic cells, characterized by a network of interconnected tubules and flattened sacs. The ER is divided into two distinct regions: the rough endoplasmic reticulum (RER), which is studded with ribosomes and primarily involved in the synthesis of proteins destined for secretion or for use in the cell membrane,

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and the smooth endoplasmic reticulum (SER), which lacks ribosomes and is associated with lipid synthesis, detoxification, and calcium ion storage.

The Golgi apparatus, also known as the Golgi complex or Golgi body, is a membranous organelle found in eukaryotic cells. It consists of a series of flattened, membrane-bound sacs called cisternae, which are organized in a stacked arrangement.

Red blood cells (RBCs), also known as erythrocytes, are primarily responsible for oxygen transport throughout the body. They are disc-shaped cells devoid of nuclei and organelles, allowing for maximum hemoglobin content.

White blood cells (WBCs), or leukocytes, are crucial components of the immune system, defending the body against infections and foreign invaders. They are larger and more complex than RBCs, containing nuclei and organelles. WBCs can be classified into five major types based on their structure and function.

Neutrophils: These are the most abundant type of WBC, accounting for 50–70% of the total WBC count. They are phagocytes, meaning they engulf and destroy bacteria and other pathogens. Neutrophils are crucial for the first line of defense against infections**.**

Lymphocytes: These cells are key players in the immune response, including both humoral and cell-mediated immunity. They are divided into three main types: B cells, T cells, and natural killer (NK) cell.

Monocytes: These are large WBCs that differentiate into macrophages upon entering tissues. Macrophages are phagocytes that engulf and destroy debris, pathogens, and foreign substances. They also play a crucial role in tissue repair and immune regulation.

Eosinophils: These cells are involved in allergic reactions and parasitic infections. They release inflammatory mediators and enzymes that help to combat these threats.

Basophils: Similar to eosinophils, basophils are involved in allergic reactions. They release histamine and other substances that contribute to inflammation and allergic symptoms.

Application of AIE Organelle-Targeted Probes

The Introduction of AIE Organelle-Targeted Probes

Cell differentiation, the process by which cells acquire distinct identities and functions, is closely linked to the unique characteristics of their organelles. These structural and functional differences among organelles provide critical insight into cellular health, disease progression, and biological diversity. Recent advancements in fluorescence imaging have made it possible to visualize these organelle variations in real time, with aggregation-induced emission (AIE)-based probes emerging as a powerful tool for such applications. AIE fluorescent probes are particularly advantageous due to their enhanced brightness in aggregated states, excellent photostability, and minimal background noise, making them ideal for targeting specific organelles in live-cell imaging.

Organelle-targeted AIE probes enable precise distinction between various cell types by exploiting differences in organelles. For example, abnormal cells, such as cancer cells, often exhibit altered mitochondrial membrane potential (MMP) and increased lipid droplet (LD) content compared to normal cells. Similarly, the structural complexity of bacterial membranes differs substantially from that of eukaryotic cells, providing another level of specificity for probe design. Even among bacteria, Gram-positive and Gram-negative species display distinct cell wall structures that can be selectively imaged using tailored probes. Furthermore, live and dead cells exhibit divergent organelle states, particularly in mitochondrial and membrane functions, which can be detected using these probes.

The ability to differentiate cells based on organelle characteristics is significant for both research and clinical applications. It allows for the identification of diseased cells, monitoring of cellular responses to treatment, and precise detection of pathogens. The role of organelle-targeted AIE fluorescent probes in distinguishing various cell types, including normal and abnormal cells,

eukaryotic cells and bacteria, different bacterial species, and live versus dead cells, will be addressed. Through the development and application of these innovative probes, researchers can gain deeper insights into cell biology and improve diagnostic and therapeutic strategies across a range of diseases.

The Principle of Organelle-Targeting

Organelle-targeted fluorescent probes leverage the unique biochemical environments, structural features, and molecular compositions of different cellular organelles to achieve specific localization. The success of organelle-targeting relies on designing probes that can selectively interact with these distinct environments, leading to high-contrast imaging and accurate differentiation of cell types. Below is a discussion of the principles behind targeting key organelles, including mitochondria, lysosomes, lipid droplets, the nucleus, the endoplasmic reticulum, and the plasma membrane.

Mitochondria Targeting: Mitochondria are central to cellular energy production and apoptosis, making them critical targets for differentiation between normal and abnormal cells, such as cancer cells. MMP is a key feature that varies significantly between healthy and diseased cells. Fluorescent probes targeting mitochondria typically exploit this difference by using cationic dyes that accumulate in the negatively charged mitochondrial matrix, such as **Mito-Probe-01**, **Mito-Probe-02**, and **Mito-Probe-03** which those based on AIEgens. These probes are highly sensitive to changes in MMP, enabling clear imaging of mitochondrial dynamics in live cells.

Lysosome Targeting: Lysosomes are acidic organelles involved in degrading cellular waste and maintaining metabolic homeostasis. Their lower pH (around 4.5–5.0) provides an ideal environment for pH-sensitive fluorescent probes and protonates these amines, increasing their lipid solubility and preventing diffusion. AIE-based lysosomal probes such as **Lyso-Probe-01** and **Lyso-Probe-02** are designed to fluoresce in the acidic lysosomal lumen, enabling the selective imaging of lysosomes. This approach is useful for distinguishing cells undergoing autophagy, detecting cancer cells, and investigating lysosomal dysfunction in diseases such as neurodegenerative disorders.

Lipid Droplet Targeting: Lipid droplets are intracellular storage organelles for neutral lipids, and their size and abundance vary significantly between normal and pathological cells. AIE probes that target LDs are designed to recognize the hydrophobic environment of these organelles, exhibiting strong fluorescence in low-polarity regions. The increased number of LDs in cancer cells and metabolic diseases, such as nonalcoholic fatty liver disease (NAFLD), makes LD-targeted imaging critical for diagnosing and understanding these conditions.

Endoplasmic Reticulum Targeting: The endoplasmic reticulum is responsible for protein synthesis and lipid metabolism, and it plays a key role in cellular stress responses. ER-targeting probes usually recognize the high calcium ion concentration or the presence of specific proteins associated with the ER membrane. AIE-based ER probes can selectively accumulate in this organelle with the help of ER targetable moiety such as p-toluenesulfonamide (**ER-Probe-01~04**) which binds the ATP-sensitive potassium (KATP) on the ER membrane.

Nucleus Targeting: Organelle-targeted probes for the nucleus typically interact with nucleic acids (DNA and RNA) through electrostatic interactions or by intercalating between DNA strands. AIE probes are particularly useful here, as their non-radiative decay is minimized in the dense nucleic acid environment, leading to bright fluorescence. This enables precise imaging of nucleus dynamics during cell division, apoptosis, and cancer progression.

Membrane Targeting: The plasma membrane and internal organelle membranes serve as barriers that regulate the flow of ions, nutrients, and signaling molecules. Targeting the membrane with fluorescent probes involves recognizing specific membrane components such as lipids, proteins, or the overall membrane potential. AIE probes can be tailored to insert into the hydrophobic membrane bilayer, providing a robust tool for visualizing membrane dynamics, tracking cell surface receptors, and identifying differences

between bacterial and eukaryotic cell membranes. This approach is valuable for differentiating bacterial pathogens, understanding cell signaling, and studying membrane-associated diseases.

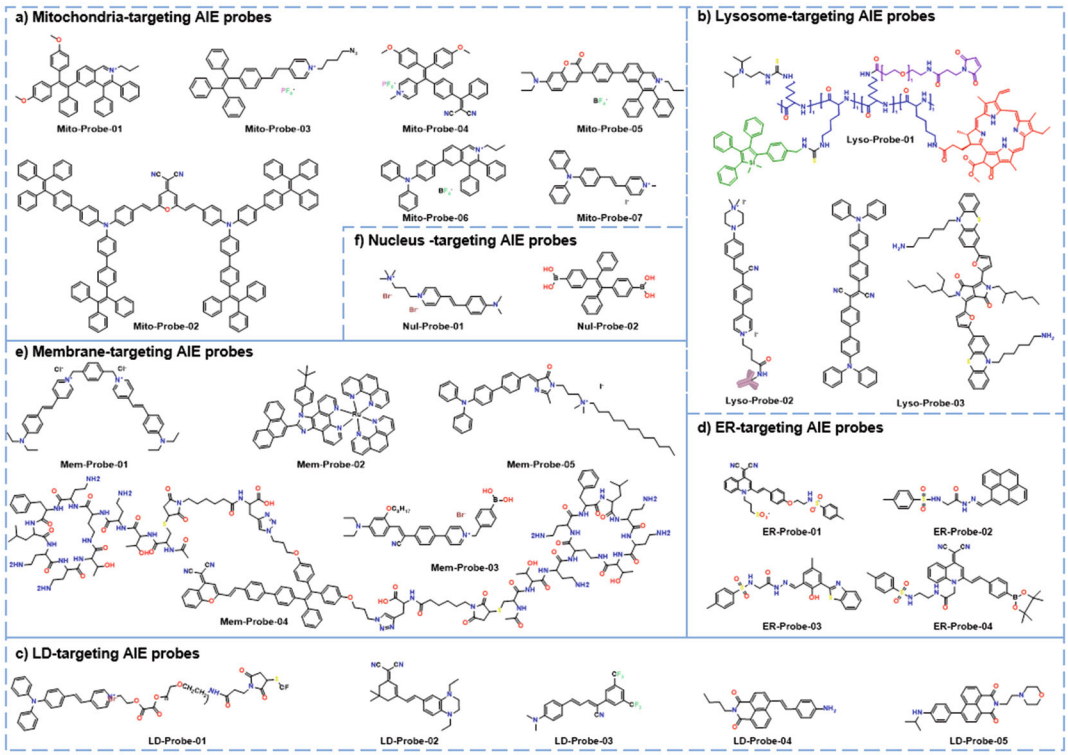
The Application of AIE Organelle-Targeted Probes in Cell Sorting

Mitochondrial-Targeting AIE Probes

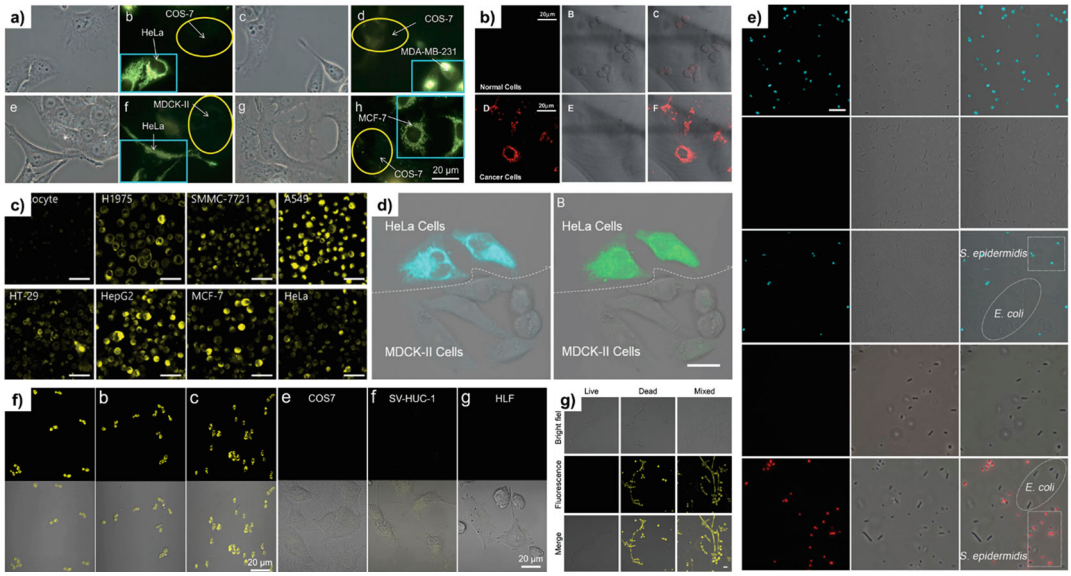
Cancer cells possess a significantly higher MMP than normal cells, with a difference of at least 60 mV due to their enhanced metabolic activity. This elevated MMP serves as a crucial biomarker, enabling fluorescent probes to differentiate cancerous cells from healthy ones. Mitochondria-specific probes, particularly those incorporating cationic AIEgens, are hypothesized to preferentially target cancer cells over normal cells.

Several AIE-based mitochondrial-targeting probes have been synthesized and evaluated (Fig. 1a) for their capacity to distinguish between cancerous and normal cells. For instance, Gui et al. demonstrated the effectiveness of AIE probes for cancer cell-specific mitochondrial imaging [1]. They synthesized **Mito-Probe-01**, an AIE dye that selectively stained the mitochondria of cancer cells while showing no staining in normal cells. This selective staining was consistent across multiple cancer cell lines, including HeLa, MCF-7, PC-9, MDA-MB-231, and A549, while normal cells such as COS-7, HEK-293, and MDCK-II remained unstained. Even in coculture systems, **Mito-Probe-01** (Figs. 1a and 2a) selectively illuminated cancer cells, highlighting its potential for imaging-guided tumor surgery.

Beyond single-photon imaging, AIE probes have also been explored for multiphoton microscopy applications. Nicol et al. developed **Mito-Probe-02** (Figs. 1a and 2b), a novel AIEgen with deep-red emission and favorable nonlinear optical



Organelle-Targeted AIE Probes for Cell Sorting, Fig. 1 The structures of selected AIE organelle-targeted probes. (**a**) Mitochondrial-targeting AIE probes; (**b**) lysosome-targeting AIE probes; (**c**) LD-targeting AIE probes; (**d**) endoplasmic-targeting AIE probes; (**e**) membrane-targeting AIE probes; (**f**) nucleus-targeting AIE probes



Organelle-Targeted AIE Probes for Cell Sorting, Fig. 2 Differentiation of cells using AIE mitochondrial-targeted probes. (**a**) Coculture of different combinations of cancer cells and normal cells in culture medium with **Mito-Probe-01**; (**b**) CLSM images of MDCK-II cells (top) and HeLa cells (bottom) after incubation with **Mito-Probe-02**; (**c**) fluorescence images of different cell types and blood leukocytes stained with **Mito-Probe-03**; (**d**) cocultured HeLa and MDCK-II cells stained with **Mito-Probe-04** (left) before and (right) after laser treatment; (**e**) selective imaging of Gram-positive bacteria. Fluorescent, bright-field, and merged images of (first row) S. epidermidis, (second row) E. coli, and (third row) a mixture of both incubated with Mito-Probe-04 for 10 min. Effective killing of Gram-positive bacteria: Fluorescent, bright-field, and merged images of a mixture of S. epidermidis and E. coli incubated (fourth row) without and (fifth row) with **Mito-Probe-04** for 10 min, followed by white light exposure for 20 min and then staining with PI; (**f**) CLSM fluorescence and merged images of C. albicans stained by **Mito-Probe-01**, **Mito-Probe-05**, and **Mito-Probe-06** and three mammalian cell types (COS7, SV-HUC-1, and HLF cells) stained with **Mito-Probe-06**, respectively; (**g**) live/dead bacterial differentiation using **Mito-Probe-07** and CLSM images of C. albicans stained with **Mito-Probe-07**

properties. Biotinylated **Mito-Probe-02** nanoparticles specifically stained mitochondria and selectively targeted live cancer cells [2].

In addition to targeting cancer cells, AIE mitochondrial-targeting probes offer a promising approach for differentiating leukocytes from circulating tumor cells (CTCs). CTCs, shed from primary tumors into the bloodstream, are vital biomarkers for cancer metastasis. Accurate detection and quantification of CTCs are essential for evaluating treatment responses and monitoring disease progression. However, the high abundance of leukocytes in blood samples complicates CTC detection. **Mito-Probe-03** (Figs. 1a and 2c), composed of TPE and pyridine azide, developed by Situ [3], selectively stains mitochondria in cancer cells while exhibiting minimal fluorescence in leukocytes. This differential fluorescence allows for the noninvasive imaging and identification of CTCs. In coculture systems, cancer cells labeled with **Mito-Probe-03** emitted bright yellow fluorescence, while leukocytes displayed weak fluorescence, enabling distinction between these cell types. The authors simulated real-world conditions by mixing cancer cells with leukocytes and successfully detected both cell types using **Mito-Probe-03**, demonstrating its potential for accurate CTC identification in complex biological samples.

AIE mitochondrial-targeting probes have also shown versatility in distinguishing various biological entities, including bacteria and fungi. Gram-negative bacteria possess a more robust barrier to foreign substances due to their complex envelope structure compared to Gram-positive bacteria. Thus, fluorescent probes are hypothesized to

preferentially penetrate Gram-positive bacteria. To test this, Kang et al. developed the mitochondrial-targeting probe **Mito-Probe-04** (Figs. 1a and 2d) and used Gram-positive S. epidermidis and Gram-negative E. coli as representative bacteria [4]. Upon incubation with **Mito-Probe-04** for 10 min, S. epidermidis exhibited blue-green fluorescence, while E. coli displayed minimal fluorescence, reflecting **Mito-Probe-04**'s lower staining efficiency for Gram-negative bacteria. In a coculture system, S. epidermidis was clearly distinguished from E. coli based on their intense blue-green fluorescence. These results suggest **Mito-Probe-04**'s potential for selectively discriminating between Gram-positive and Gram-negative bacteria through fluorescence imaging techniques. Furthermore, **Mito-Probe-04** was capable of simultaneously imaging cancer cells and Gram-positive bacteria (Fig. 2f). In coculture with HeLa cells, MDCK-II normal cells, and S. epidermidis, both HeLa cells and S. epidermidis exhibited blue-green fluorescence, whereas MDCK-II cells lacked fluorescence signals.

Additionally, cationic AIE mitochondrial probes have demonstrated selective targeting of fungal mitochondria over mammalian cells due to the more negative MMP in fungi. Three AIEgens—**Mito-Probe-01**, **Mito-Probe-05**, and **Mito-Probe-06** (Figs. 1a and 2e)—containing cationic isoquinolinium (IQ) moieties with appropriate hydrophobicity, preferentially targeted fungal mitochondria [5].

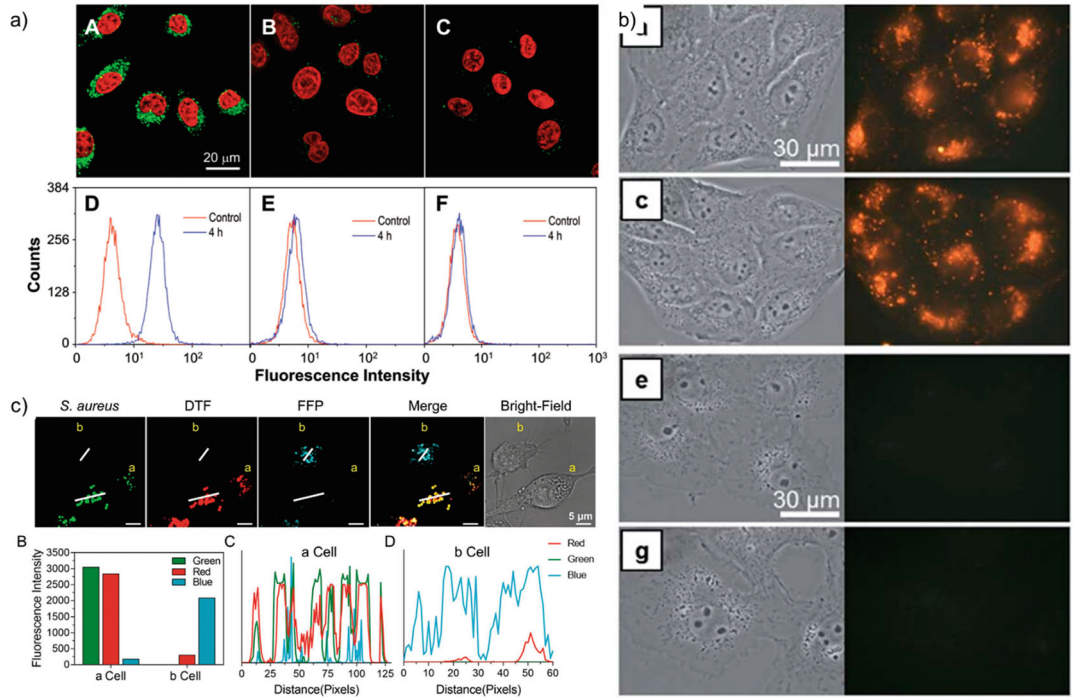
Moreover, mitochondria-targeting probes can distinguish between live and dead cells. Ge et al. developed **Mito-Probe-07** (Figs. 1a and 2g) [6], a probe that fluoresced in dead fungal cells but showed minimal fluorescence in viable ones, providing a method for assessing cell viability.

Lysosome-Targeting AIE Probes

Lysosomes, essential organelles for cellular processes such as protein secretion and waste disposal, have become promising targets for cancer diagnosis and drug delivery due to their unique acidic environment (pH 4.5–5.0). Precise lysosome tracking is critical for understanding their role in cancer development and treatment. Yuan et al. demonstrated the potential of AIE probes for cancer cell differentiation [7]. Their probe, **Lyso-Probe-01** (Fig. 1b), composed of tetraphenylsilole (TPS) and pheophorbide A (PheA), exhibited strong fluorescence specifically in integrin-positive MDA-MB-231 breast cancer cells compared to integrin-negative MCF-7 and 293 T cells (Fig. 3a). This selective targeting enables efficient cancer cell identification.

Beyond targeting cancer cells, AIE probes can be further enhanced for specific target recognition. Monoclonal antibodies (mAbs) are known for their high target specificity, making them ideal candidates for conjugation with AIE probes to create "turn-on" fluorescent probes for wash-free cancer cell imaging. Shi et al. [8] developed a mAb-AIE probe conjugate (**Lyso-Probe-02**; Fig. 1b) that exhibits weak fluorescence in solution due to non-radiative decay. However, upon internalization into EGFR-positive cancer cells via EGFR-mediated endocytosis, the conjugate becomes highly emissive. This "turn-on" effect results from the lysosomal hydrolysis of the **Lyso-Probe-02**, generating cationic AIE metabolites that accumulate in mitochondria and emit strong light. Importantly, the **Lyso-Probe-02** conjugate displayed a high target-to-background ratio due to the minimal background signal in non-cancer cells, allowing for clear differentiation of tumors from healthy tissues (Fig. 3b).

Another exciting application of AIE probes lies in the detection of bacterial infected cells. Hypochlorous acid (HClO) production within lysosomes of phagocytes serves as a biomarker for bacterial infection. Wu et al. [9] developed an AIE HClO probe (**Lyso-Probe-03**; Fig. 1b) that visualizes HClO in bacteria-infected cells. This probe exhibits bright red fluorescence specifically in S. aureus-infected RAW 264.7 cells, contrasting with the near-infrared fluorescence observed in noninfected cells. This selective response enables sensitive discrimination between infected and healthy cells (Fig. 3c). More importantly, the **Lyso-Probe-03** probe can also generate reactive oxygen species (ROS) within infected cells, leading to the destruction of bacteria and infected phagocytes with minimal side effects on healthy cells.



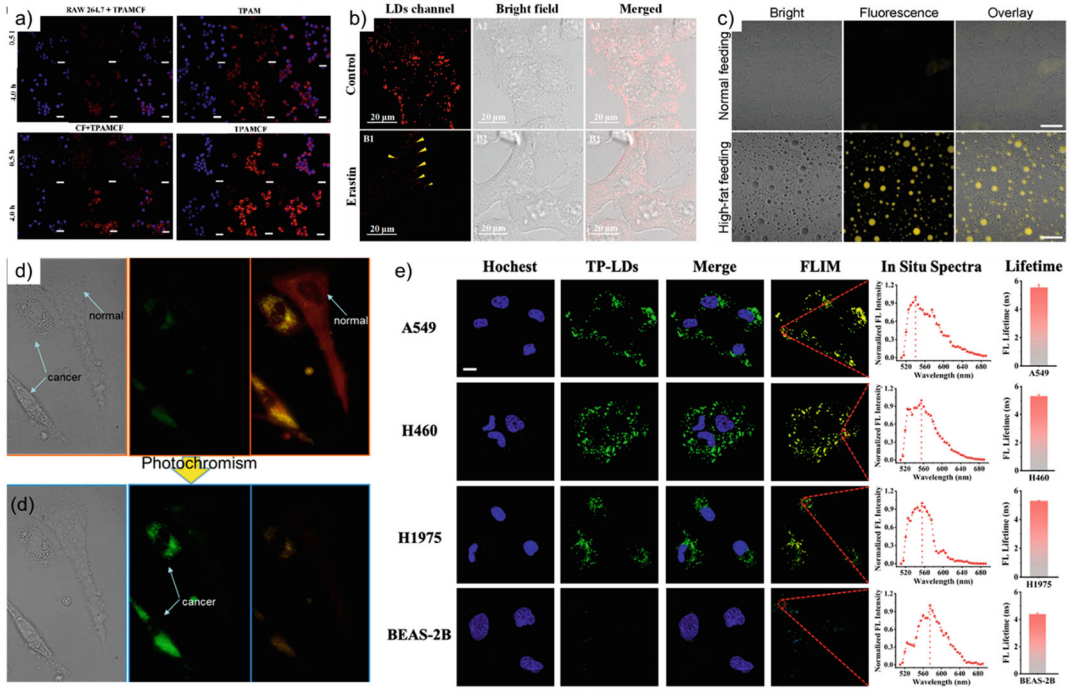
Organelle-Targeted AIE Probes for Cell Sorting, Fig. 3 Differentiation of cells using AIE lysosome-targeting probes. (**a**) Differentiation of breast cancer cells from other cell types using **Lyso-Probe-01**; (**b**) bright-field and fluorescent images of HCC827 cancer cells and COS-7 normal cells incubated with **Lyso-Probe-02**. Images were acquired (first and third rows) in probe medium without washing and (second and fourth rows) in pure medium after washing; (**c**) confocal fluorescence images of live RAW 264.7 cells treated with **Lyso-Probe-03**, followed by treatment with S. aureus for 1 hour. Green fluorescence indicates SYTO 9-stained S. aureus; red fluorescence indicates **Lyso-Probe-03**. Fluorescence intensity analysis and line-scan profiles of bacteria-infected and noninfected cells and differentiation of infected cells from normal cells using **Lyso-Probe-03**

LD-Targeting AIE Probes

Lipid droplets (LDs) are dynamic cellular organelles that store neutral lipids, including triglycerides and cholesterol esters. They play pivotal roles in energy metabolism, lipid homeostasis, and cellular signaling. Notably, the size, number, and composition of LDs can significantly differ between normal and pathological cells, making them valuable biomarkers for various diseases.

In atherosclerotic plaques, the accumulation of LDs within macrophages and smooth muscle cells is a hallmark of plaque development. These intracellular lipid deposits exacerbate inflammation and oxidative stress, potentially leading to plaque rupture and cardiovascular events. To address this, lipid-targeting AIE probes have emerged as a promising diagnostic tool. During atherogenesis, macrophages internalize oxidized low-density lipoprotein (LDL), transforming into foam cells, which are a key target for atherosclerotic plaque detection. Liu et al. developed **LD-Probe-01** (Fig. 1c), an AIE probe that composed of an AIEgen, a ROS-responsive linkage, and a CF peptide (CLIKKPF) [10]. **LD-Probe-01** accumulates in atherosclerotic plaques through CF's high affinity for externalized phosphatidylserine on apoptotic foam cells, and in vitro evaluations demonstrated **LD-Probe-01**'s foam cell targeting ability (Fig. 4a).

In liver diseases, such as nonalcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease, excessive lipid droplet (LD) accumulation within hepatocytes leads to liver dysfunction and inflammation. Hong et al. developed **LD-Probe-02** (Fig. 1c), a high-fidelity AIE-active probe that is highly sensitive to polarity and exhibits strong



Organelle-Targeted AIE Probes for Cell Sorting, Fig. 4 Differentiation of cells using AIE lipid Droplet-Targeting Probes. (**a**) CLSM images of RAW 264.7 cells (top) and foam cells (bottom) stained with **LD-Probe-01**; (**b**) CLSM imaging of lipid droplets in a ferroptosis cell model (induced with 20 μM Erastin for 12 hours) (top) and HeLa cells (bottom) with **LD-Probe-02**; (**c**) fluorescence images of normal and high-fat diet-induced nonalcoholic fatty liver disease (NAFLD) guinea pig liver tissues stained with **LD-Probe-03**; (**d**) cocultured MRC-5 normal lung fibroblast cells and A549 lung cancer cells before (top) and after (bottom) photochromism with **LD-Probe-04**; (**e**) imaging of **LD-Probe-05** in lung cancer cells and normal cells

fluorescence under conditions of extreme low polarity [11]. This feature enhances **LD-Probe-02**'s fluorescence in NAFLD cells, which have increased size and number of LDs compared to normal cells, enabling clear differentiation between NAFLD and normal liver tissues in 3D imaging (Fig. 4b). To enhance the clinical applicability of fluorescent probes, Park et al. reported **LD-Probe-03** (Fig. 1c), a two-photon AIE probe designed for diagnosing NAFLD in deep tissues [12]. **LD-Probe-03**'s deeper tissue penetration represents a significant advancement in improving the diagnostic capabilities of NAFLD (Fig. 4c).

Cancer cells exhibit elevated lipid droplet (LD) levels compared to normal cells, primarily due to the Warburg effect [13], which enhances glycolysis and the production of pyruvate, a precursor of fatty acids and lipogenic molecules. Taking advantage of that, Lai et al. developed **LD-Probe-04** (Fig. 1c), an AIE probe specifically targeting LDs for cancer cell diagnosis [14]. **LD-Probe-04** exhibits high specificity for lipid droplets (LDs) and demonstrates distinct behavior under irradiation in cancer cells compared to normal cells (Fig. 4d). In cancer cells, **LD-Probe-04** undergoes photochromism, while normal cells experience photobleaching. This phenomenon enables the differentiation of cancer and normal cells using **LD-Probe-04**, and the staining of five cancer cell lines and three normal cell lines confirmed this observation. Additionally, coculture experiments revealed that bright spots appeared exclusively in LDs of cancer cells under irradiation. These distinct cellular staining patterns suggest that **LD-Probe-04**, in conjunction with appropriate filters, can be used to effectively differentiate cancer cells from normal cells. Another

difference of lipid droplet between normal cell and cancer cell is the microenvironment, especially polarity. Pu et al. reported an AIE-active two-photon fluorescent probe, **LD-Probe-05** (Fig. 1c), with a D-π-A structure for tracking LD polarity variations in different cells [15]. In normal cells, the fluorescence intensity was lower, the emission wavelengths were longer, and the fluorescence lifetime was significantly reduced compared to cancer cells (Fig. 4e). These findings are consistent with polarity measurements in solution, indicating a distinct difference in LD polarity between normal and cancer cells. And a multimodal approach considering fluorescence intensity, maximum emission wavelength, and fluorescence lifetime can significantly improve the accuracy of cell differentiation compared to relying solely on fluorescence intensity.

Endoplasmic Reticulum-Targeting AIE Probes

The endoplasmic reticulum (ER) is a pivotal organelle within eukaryotic cells, involved in a multitude of essential cellular processes. Its functions encompass protein synthesis, folding, and trafficking, as well as calcium homeostasis, lipid synthesis, and cell signaling. In normal cells, the ER operates seamlessly to ensure the proper production and function of proteins. However, disruptions in ER function can lead to a cascade of problems, contributing to a variety of diseases. For example, impaired protein folding can result in the accumulation of misfolded proteins, triggering ER stress, which has been implicated in conditions like Alzheimer's disease and diabetes. Additionally, abnormal ER calcium signaling has been associated with neurological disorders.

While numerous AIE probes have been developed for targeting the ER, these probes often lack the ability to differentiate between normal and abnormal cells. For instance, **ER-Probe-01** (Fig. 1d), developed by Zhu et al. [16], is a notable example of an ER-targeting AIE probe. By incorporating a p-toluenesulfonamide moiety, **ER-Probe-01** enhances its lipophilicity and is capable of targeting the KATP channel on the ER membrane, generating targeted AIE signals. However, despite these advantages, **ER-Probe-01** is unable to distinguish between healthy and diseased cells.

However, recent advancements in fluorescent probe development have showcased significant potential in distinguishing between normal and abnormal cells. One such promising probe is **ER-Probe-02,ER-Probe-03** [17, 18], developed by Yan et al. This ER-targeted probe not only efficiently targets the ER but also enables ratiometric fluorescence imaging of ClO− ions within the ER. As ClO− ion levels are elevated under ER stress conditions, **ER-Probe-02,ER-Probe-03** (Fig. 1d) can be used to visually differentiate between normal cells and those experiencing ER stress.

Similarly, ER stress plays a pivotal role in drug-induced liver injury (DILI), where peroxynitrite (ONOO-) is a key biomarker. To address this, Zhang et al. developed **ER-Probe-04** (Fig. 1d), a novel ER-targeted near-infrared fluorescent probe with AIE properties [19]. **ER-Probe-04** has been successfully applied for both in vitro and in vivo imaging of endogenous ONOO− fluctuations. With its excellent ER-targeting capability, large Stokes shift, and low detection limit, **ER-Probe-04** can effectively visualize fluctuations of both exogenous and endogenous ONOO− in HepG2 cells. Importantly, **ER-Probe-04** exhibits relatively low cytotoxicity, making it a promising tool for differentiating drug-induced liver-injured cells from normal cells.

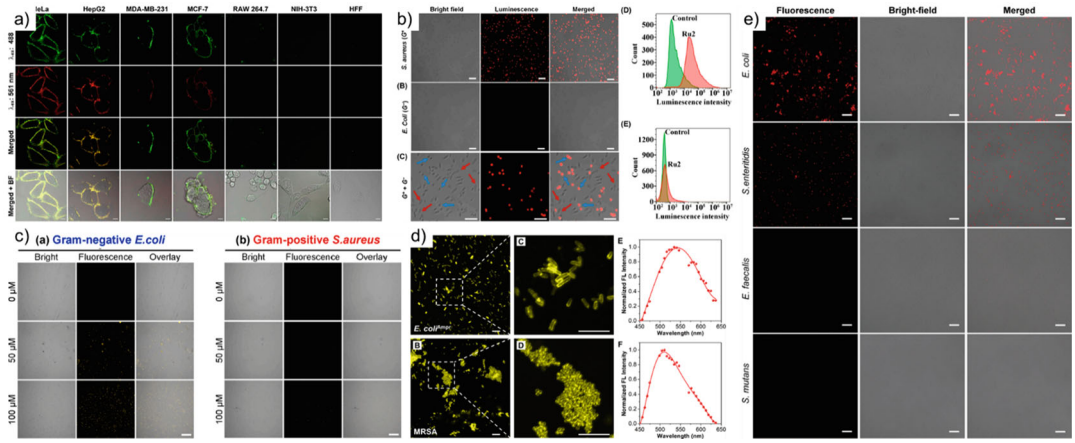
Cell Membrane-Targeting AIE Probe

The cell membrane, a vital component of cellular architecture, serves as a selective barrier regulating the passage of molecules into and out of the cell. In normal cells, the membrane's phospholipid bilayer, interspersed with proteins, cholesterol, and glycolipids, maintains a precise balance of permeability. However, in abnormal cells, deviations from this standard composition and function can significantly impact cellular health. Alterations in lipid composition, protein expression, or membrane fluidity may lead to impaired cell signaling, altered nutrient uptake, and increased vulnerability to damage. These aberrations can contribute to a wide range of pathological conditions, including cancer, neurodegenerative diseases, and metabolic disorders.

Wu et al. developed the novel probe **Mem-Probe-01** (Fig. 1e) for the selective visualization of cancer cells based on their distinct plasma membrane (PM) lipid composition [20]. **Mem-Probe-01**'s folded structure confers exceptional PM retention properties, enabling long-term PM tracking for 6 hours without significant signal loss or labeling of other subcellular compartments (Fig. 5a). Moreover, **Mem-Probe-01** can reliably distinguish cancer cells from non-cancer cells in both live and fixed cell preparations. After staining various cancer cell lines (HeLa, HepG2, MDA-MB-231, and MCF-7) with 2.0 mM **Mem-Probe-01** for 30 min at 37 °C, the PM of all cancer cells was readily visualized under 488 or 561-nm excitation. In contrast, normal cells, including mouse macrophages (RAW 264.7), mouse embryo fibroblasts (NIH 3 T3), and human foreskin fibroblasts (HFFs), exhibited no fluorescence. These findings demonstrate the potential of **Mem-Probe-01** as a valuable tool for cancer cell identification and imaging.

In addition to distinguishing normal cells, membrane-targeted fluorescent probes are powerful tools for distinguishing between different classes of bacteria, particularly those with distinct membrane structures. Bacteria are primarily classified into two groups based on their cell wall composition: Gram-positive (G+) and Gram-negative (G−). The former is characterized by a thick peptidoglycan layer, while the latter features a thinner peptidoglycan layer and an outer membrane composed of lipopolysaccharides (LPS) and lipoprotein.

The unique outer wall structures of bacteria significantly influence their binding interactions with aggregation-induced emission (AIE)-active molecules, enabling discrimination through AIEgens. For instance, a ruthenium-based AIEgen complex, **Mem-Probe-02** (Fig. 1e), developed by Liu et al. [21], demonstrates selective imaging of G+ bacteria due to its strong binding affinity with the thick peptidoglycan layer of G+ bacteria. This selective recognition is achieved through a washing-free staining procedure, highlighting the potential of AIEgens in bacterial discrimination (Fig. 5b). However, the presence of an additional outer membrane in G− bacteria poses a challenge



Organelle-Targeted AIE Probes for Cell Sorting, Fig. 5 Differentiation of cells using AIE membrane-targeting probes. (**a**) Images of the plasma membrane (PM) stained with **Mem-Probe-01** in cancer cells (HeLa, HepG2, MDA-MB-231, and MCF-7) and non-cancer cells (RAW 264.7, NIH 3 T3, and HFF); (**b**) CLSM images of Gram-positive S. aureus (indicated by blue arrows) and Gram-negative E. coli (indicated by red arrows) treated with **Mem-Probe-02** and flow cytometric results quantifying the fluorescence intensities of S. aureus and E. coli before (control) and after treatment with **Mem-Probe-02**; (**c**) confocal fluorescence images of Gram-negative E. coli and Gram-positive S. aureus incubated with **Mem-Probe-03** at different concentrations; (**d**) CLSM images of E. coli, S. enteritidis, E. faecalis, and S. mutans incubated with **Mem-Probe-04**; (**e**) CLSM images of Gram-negative E. coli and Gram-positive methicillin-resistant S. aureus (MRSA) after incubation with **Mem-Probe-05** and in situ emission spectra extracted from the panels

for fluorescent probes, as it hinders their penetration and staining of these bacteria. To address this, strategies targeting the LPS component of the G− bacteria's outer membrane have been developed to enhance the staining of G− bacteria. For instance, **Mem-Probe-03** (Fig. 1e), a fluorescent AIE probe with high photostability, was designed for selective LPS detection [22], enabling high-contrast discrimination of G− bacteria over G+ bacteria (Fig. 5c). Additionally, **Mem-Probe-04** (Fig. 1e), which also targets LPS, has been developed by Bao et al. [23], leveraging the strong binding effect between polymyxin B and LPS (Fig. 5d).

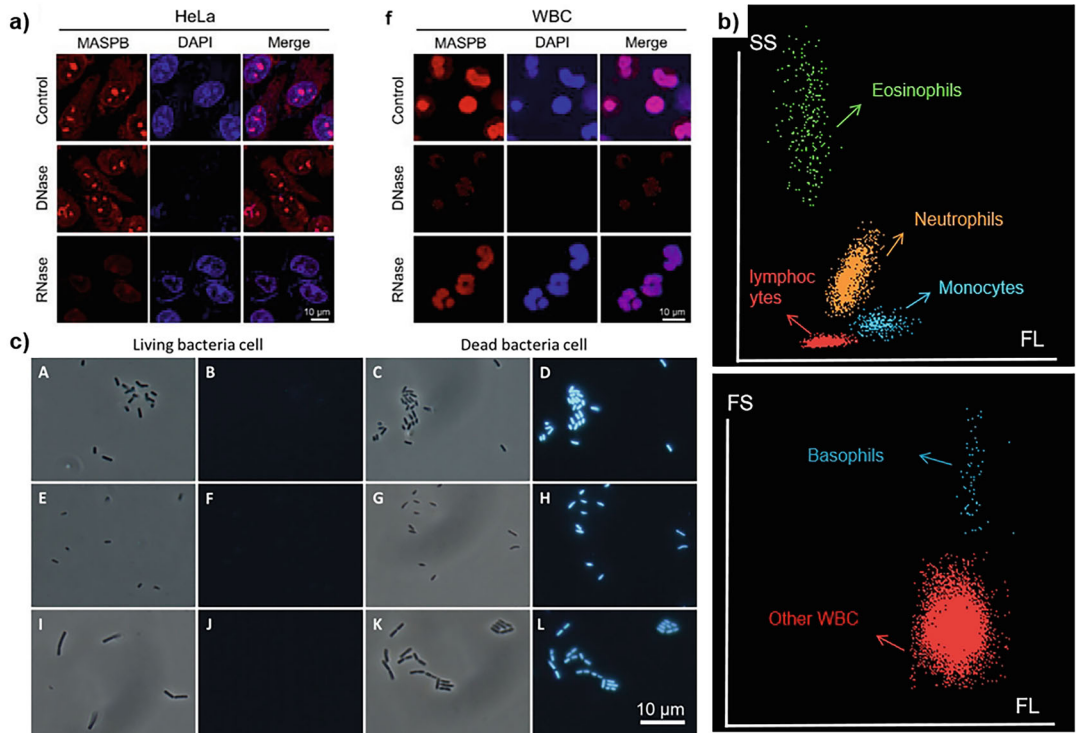
Recently, a new strategy based on polarity-sensitive fluorescent probes was developed by Wang et al. [24]. **Mem-Probe-05** (Fig. 1e), a membrane-targeting probe, was designed to reflect the packing degree of bacterial membrane lipids (Fig. 5e). This probe, composed of a polarity-sensitive emissive unit with TICT and AIE properties, exhibits a significant spectral shift in response to different solvent polarities. Upon incubation with different types of bacteria, **Mem-Probe-05** displayed differential spectral shifts, with G+ bacteria showing a more pronounced blue shift compared to G− bacteria, indicating a spectral gap of approximately 30 nm. This difference is attributed to the probe's intercalation into the densely packed cell membrane of G+ bacteria, creating a hydrophobic environment, versus its localization in the loosely packed outer membrane of G− bacteria, generating a less hydrophobic environment. **Mem-Probe-05**'s sensitivity to polarity changes allows for the rapid differentiation of G− and G+ bacteria, despite subtle differences in membrane lipid packing.

Nucleus-Targeting AIE Probes

The nucleus, the control center of the cell, houses the genetic material and regulates cellular activities. In normal cells, the nucleus is typically round or oval-shaped, with a well-defined nucleus envelope and evenly distributed chromatin. However, in abnormal cells, deviations from this standard morphology can be indicative of pathological processes. For instance, enlarged nuclei, irregular shapes, or condensed chromatin can be associated with cancer cells. These nucleus alterations often reflect changes in gene expression, cell cycle regulation, or DNA damage. Additionally, for the white blood cells, or leukocytes, could be dived into five catalogues based on the nucleus shape. The most prevalent of these cells, neutrophils, are equipped with a multilobed nucleus that facilitates their rapid migration to infection sites. Lymphocytes, vital for antibody production and immune signaling, possess a large, round nucleus that occupies a significant portion of the cell. Monocytes, which mature into macrophages, feature a bean-shaped nucleus, while basophils and eosinophils, key players in allergic responses and parasitic infections, are distinguished by their bilobed nuclei.

AIE fluorescent probes often interact with nucleic acids through various mechanisms, including intercalation, groove binding, and electrostatic interactions. Intercalation involves the insertion of the AIE probe between base pairs of DNA or RNA, disrupting the base stacking interactions. Groove binding occurs when the AIE probe binds to the major or minor groove of the nucleic acid helix. Electrostatic interactions between charged AIE probes and the negatively charged phosphate backbone of nucleic acids can also contribute to binding. The specific binding mode of an AIE probe depends on its molecular structure, the type of nucleic acid, and the solution conditions. And Situ et al. fount that **Nul-Probe-01** (Fig. 1f) could rapidly diffuse into the cell, and once penetrating into the condensed nucleus structures, the intramolecular motions of **Nul-Probe-01** were confined to varying extent, depending on the different condensation of nucleus [25]. As a result, the nucleus density could be monitored by **Nul-Probe-01**, and the distributions of **Nul-Probe-01** in tumor cells were distinct from those of WBC (Fig. 6a). The ability to distinguish between these leukocyte types is further enhanced by the use of AIE nucleus-targeting probe-based Blood Cell Analysis Kit, causing them to emit fluorescence signals. By employing flow cytometry to collect this fluorescence and scattered light signals, white blood cells can be accurately classified into their respective types: lymphocytes, monocytes, neutrophils, eosinophils, and basophils (Fig. 6b).

Beyond sorting and identifying tumor cells and leukocytes, nucleus probes can be employed to



Organelle-Targeted AIE Probes for Cell Sorting, Fig. 6 Differentiation of cells using AIE nucleus-targeting probes. (**a**) Fluorescence images of HeLa cells and white blood cells (WBCs) treated with DNase or RNase, followed by staining with **Nul-Probe-01** and DAPI. (**b**) WBC differentiation using the AIE Blood Cell Analysis Kit; (**c**) bright-field and fluorescent images of dead and living E. coli (first row), S. epidermidis (second row), and B. subtilis (third row) stained with **Nul-Probe-02**

assess cellular activity. Due to their limited cell membrane penetration, most AIE nucleus probes are unable to stain normal cells or cells with low activity, such as apoptotic or dead cells. However, the compromised membrane structure of these cells facilitates probe penetration and binding to nucleic acids. In addition to normal cell viability assessment, this strategy can be extended to assess microbial viability. For example, Zhao et al. developed **Nul-Probe-02** (Fig. 1f), an impermeable dye that selectively images dead bacteria by penetrating the compromised membrane and interacting with DNA within the protoplasm (Fig. 6c) [26].

Future Aspect

As research progresses, we can anticipate significant advancements in several key areas.

1. Advances in probe design are expected to enhance targeting specificity and sensitivity. By optimizing the molecular structure and functional groups of AIE probes, researchers can develop molecules with even greater selectivity for specific organelles and stronger fluorescence signals, facilitating more precise and accurate cell sorting based on organelle characteristics.
2. The integration of AIE probes with other technologies, such as microfluidics and flow cytometry, will facilitate the development of automated high-throughput cell sorting platforms. Microfluidic devices can be used to manipulate and sort cells in a controlled environment, while flow cytometry can be combined with AIE probes to analyze the fluorescence signals of individual cells. This integration will enable rapid and efficient

sorting of large numbers of cells, making AIE probes a valuable tool for various applications.
3. The application of AIE probes in clinical settings could revolutionize cell therapy, drug discovery, and personalized medicine. By isolating specific cell populations based on their organelle characteristics, AIE probes can enable the development of more targeted and effective treatments. For example, AIE probes could be used to isolate stem cells for regenerative medicine, identify cancer cells for targeted therapy, or isolate immune cells for immunotherapy.
4. The combination of AIE probes with other imaging techniques, such as super-resolution microscopy, could provide even greater insights into cellular heterogeneity and organelle dynamics. Super-resolution microscopy allows for the visualization of structures at the nanoscale, enabling researchers to study the interactions between organelles and their role in cellular processes. This information can be used to develop more sophisticated cell sorting strategies and improve our understanding of disease mechanisms. Furthermore, as fluorescent intensity can be influenced by probe concentration, technologies that are independent of concentration should be considered for cell sorting in complex environments.

In conclusion, the future of AIE organelle-targeted probes in cell sorting is bright. As research continues to advance, we can expect to see significant improvements in probe design, integration with other technologies, and clinical applications. These advancements will have a profound impact on various fields, including cell biology, medicine, and biotechnology.

Cross-References

- ▶ AIEgens for Organelles Imaging
- ▶ Mammalian Cell Imaging and Tracking
- ▶ Microbe Imaging and Killing

Competing Interest Declaration The author(s) has no competing interests to declare that are relevant to the content of this manuscript.

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