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Article

Keywords:

Posted Date: October 26th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11188982/v1>

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Additional Declarations: There is **NO** Competing Interest.

NHC-Modulated Fe (II) Complex for TADF-Assisted Multiphoton Lifetime Imaging: Concurrent Apoptosis Monitoring and Quantification

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ABSTRACT: The application of luminescent iron complexes in bioimaging is severely constrained by ultrafast excited-state deactivation and cellular background interference. Herein, we present $[\text{Fe}(\text{imp})_2]^{2+}$, the first Fe (II) complex that enables the emergence of both three-photon excited fluorescence (3PEF) and thermally activated delayed fluorescence (TADF), accessed via stepwise substitution of terpyridine with N-heterocyclic carbene (NHC) ligands to circumvent the intrinsic drawbacks of traditional Fe-based materials. Mechanistically, the enhanced ligand field reshapes the relative energetics of the metal-to-ligand charge transfer (MLCT) and metal-centered (MC) state manifolds, disfavoring MC-mediated nonradiative decay and opening a thermally activated emissive pathway. More significantly, $[\text{Fe}(\text{imp})_2]^{2+}$ serves as an apoptosis-related spatiotemporal cascade-targeting agent, sequentially targeting the plasma membrane, mitochondria, and the entire cell. The TADF-assisted lifetime-resolved signatures render $[\text{Fe}(\text{imp})_2]^{2+}$ capable of distinguishing apoptosis from mixed cell death, with a global lifetime evolution spanning 1.1-2.4 ns and a pronounced lifetime heterogeneity of 0.43 ns. Co-incubation of $[\text{Fe}(\text{imp})_2]^{2+}$ with anti-apoptotic agents demonstrated that effective cytoprotection is achieved when the probe lifetime dropped below 1.2 ns. This work pioneers NHC-regulated Fe (II) complexes TADF-mediated lifetime probes, offering a new paradigm for transition-metal bioimaging.

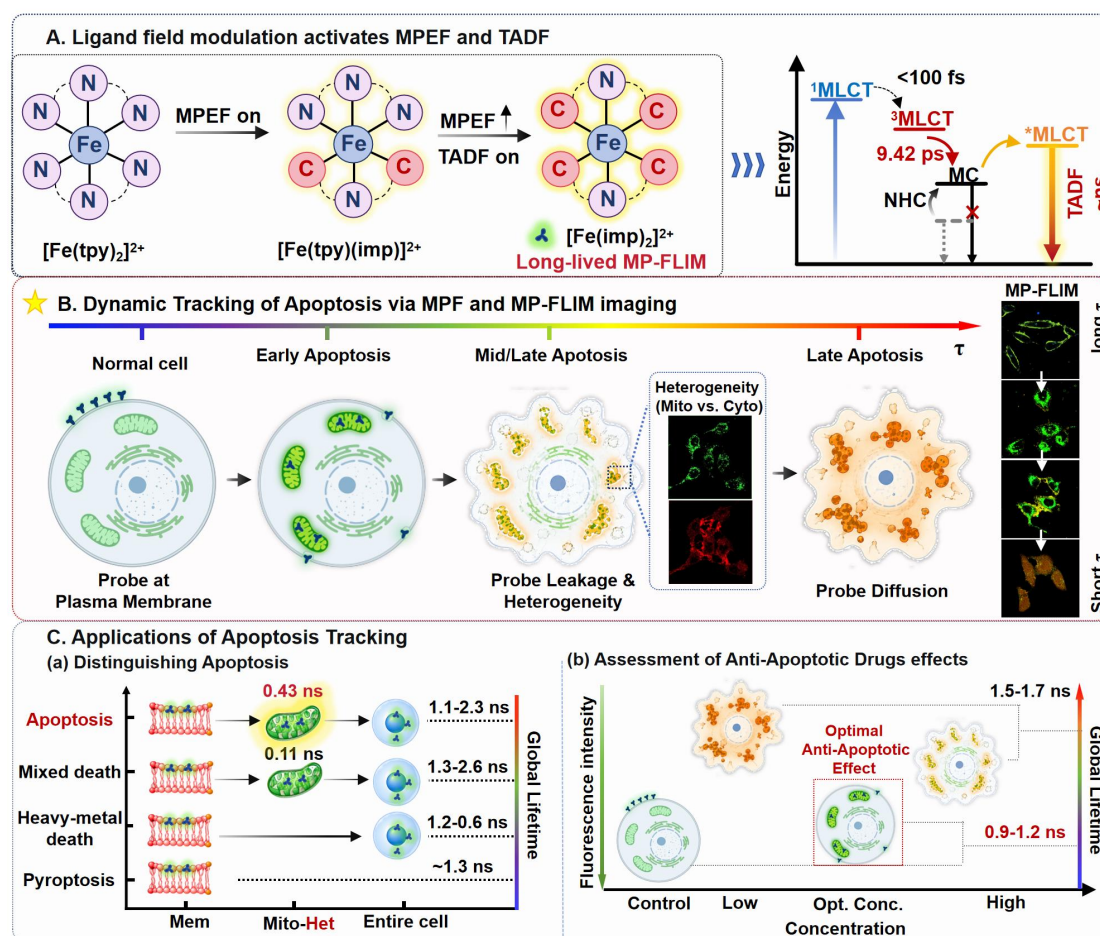
■ INTRODUCTION

Luminescent iron complexes are increasingly recognized as promising alternatives to noble-metal luminophores^[1-3]. Recent developments in luminescent iron complexes have expanded their photofunctional applications to near-infrared photosensitization, photoredox catalysis, and photon up conversion^[4-11]. Heinze and co-workers demonstrated that the cis isomer of a tetracarbene Fe (II) complex exhibits a prolonged charge transfer state lifetime in the nanosecond regime at 77K^[12]. Berkefeld and co-workers reported a cyclometalated Fe (II) luminophore that established a rare near-infrared emission through double cyclometallation^[5]. Wenger's group elegantly tuned the excited states of luminescent Fe-carbene complexes, enabling molecular-iron-sensitized triplet-triplet annihilation up-conversion^[8, 11, 13]. However, luminescent iron complexes remain largely absent from modern bioimaging. In conventional Fe (II) systems, low-lying MC states provide efficient nonradiative channels for ultrafast MLCT-to-MC conversion, resulting in weak emission and femtosecond-to-picosecond excited-state lifetimes^[14-18]. Campagna's group proposed a promising strategy to address this long-standing challenge: the thermal repopulation of the emissive Fe-centered charge-transfer state via reversible intramolecular electron transfer, thereby extending the luminescence lifetime through a thermally activated pathway^[19]. In this context, integrating Fe-based luminophores with rational excited-state dynamics regulation may enable the translation of intrinsically short-lived iron emission into quantitative lifetime-resolved bioimaging.

Apoptosis is a highly attractive target for high-precision imaging, but quantitative monitoring remains challenging due to the complexity of the involved cellular events^[20-22]. Recent studies have exploited polarity-dependent emission, lysosomal morphology, or stimulus-induced organelle redistribution to visualize apoptotic progression rather than merely detecting a terminal endpoint^[23-28]. However, current apoptosis-tracking approaches still largely rely on fluorescence intensity, emission color, or single biochemical markers. Such readouts are susceptible to variations in probe concentration, intracellular accumulation, photobleaching, local aggregation, and overlapping responses from different cell-death pathway^[20, 29-32]. The development of apoptosis probes should move beyond conventional "turn-on" fluorescence toward lifetime-resolved and spatially trackable readouts, which offer the advantage of circumventing background interference for precise quantification^[33-36]. Owing to their biological relevance, low cost, and potentially favorable biocompatibility, luminescent Fe complexes may provide effective alternatives to conventional apoptosis-detection reagents^[1, 2, 37]. Therefore, a Fe-based luminophore with prolonged delayed emission can integrate subcellular redistribution and lifetime evolution into a quantitative MP-FLIM platform for apoptosis monitoring.

In this study, we design and synthesize a series of NHC-modulated luminescent Fe complexes ($[\text{Fe}(\text{tpy})_2]^{2+}$, $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$, and $[\text{Fe}(\text{imp})_2]^{2+}$) for dynamic apoptosis profiling. The strengthened NHC-coordination suppresses MC-mediated nonradiative deactivation, transforming the intrinsically weakly emissive Fe (II) system into a multiphoton-excitable emitter with a TADF-assisted nanosecond-scale fluorescence lifetime. Among them, $[\text{Fe}(\text{imp})_2]^{2+}$

stands out as a powerful probe for high-resolution quantitative tracking of subcellular apoptotic events, capitalizing on its superior MPEF and extended lifetime to achieve high-contrast imaging with minimized background interference. The fluorescence lifetime response of $[\text{Fe}(\text{imp})_2]^{2+}$ enables precise discrimination of apoptotic from other cell death. Leveraging quantitative decoding of apoptosis-associated subcellular dynamics, $[\text{Fe}(\text{imp})_2]^{2+}$ represents a promising platform for assessing the cytoprotective effects of anti-apoptotic agents. The findings demonstrate that the enhanced ligand field endows Fe (II) complexes with nanosecond emission lifetimes and multiphoton-excitable character, establishing a competitive platform for quantitative bioimaging.



Scheme 1 N-heterocyclic carbene modulates the luminescence of Fe complexes for dynamically visualizing apoptosis.

RESULTS AND DISCUSSION

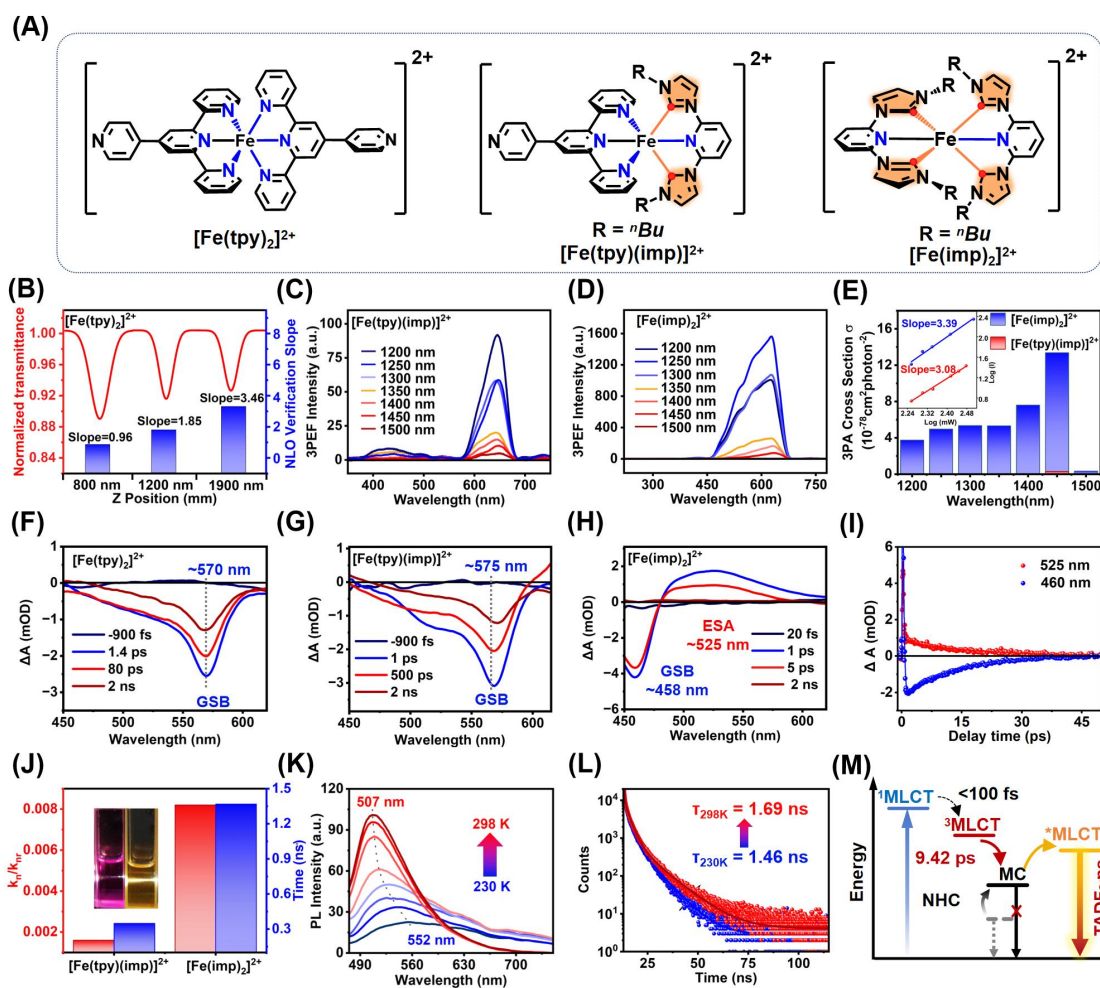


Figure 1. (A) Design strategy of Fe complexes based on stepwise N-heterocyclic carbene (NHC) modifications. (B) Multiphoton absorption performance and experimental validation of $[\text{Fe}(\text{tpy})_2]^{2+}$ (1 mM in MeCN) at excitation wavelengths of 800, 1200, and 1900 nm. (C) Three-photon excited fluorescence spectra of $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$ at excitation wavelengths of 1200–1500 nm (1 mM in MeCN). (D) Three-photon excited fluorescence spectra of $[\text{Fe}(\text{imp})_2]^{2+}$ at excitation wavelengths of 1200–1500 nm (1 mM). (E) Three-photon absorption cross section of $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$ and $[\text{Fe}(\text{imp})_2]^{2+}$. Inset: Three-photon verification of $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$ and $[\text{Fe}(\text{imp})_2]^{2+}$. Transient absorption spectra of (F) $[\text{Fe}(\text{tpy})_2]^{2+}$, (G) $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$, and (H) $[\text{Fe}(\text{imp})_2]^{2+}$ in MeCN excited at 385 nm. (I) The decay-associated spectra generated by global analysis. (J) The fluorescence lifetimes and the ratios of radiative to non-radiative rate constants (k_r/k_{nr}) of $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$ and $[\text{Fe}(\text{imp})_2]^{2+}$. (K) Temperature-dependent photoluminescence spectra of $[\text{Fe}(\text{imp})_2]^{2+}$ in acetonitrile from 230 to 298 K. (L) Temperature-dependent fluorescence lifetimes of $[\text{Fe}(\text{imp})_2]^{2+}$ in acetonitrile from 230 to 298 K. (M) Mechanistic insights into NHC-modulated luminescence properties of Fe (II) complexes.

Ligand-Field Modulation Activates MPEF and TADF in Fe Complexes

We strategically strengthen the ligand field by incorporating strong σ -donor NHC, yielding $[\text{Fe}(\text{tpy})_2]^{2+}$, $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$ and $[\text{Fe}(\text{imp})_2]^{2+}$ (Figure 1A and Figure S1-12). This substantial increase in d-orbital splitting suppresses MC-state-dominated non-radiative transitions and favors forming of long-lived MLCT excited states. Such favorable excited-state characteristics are essential to realize appropriate luminescence efficiency for Fe-based complexes^[38-41]. $[\text{Fe}(\text{tpy})_2]^{2+}$ exhibits typical reverse saturation absorption (RSA) signals (Figure 1B) under 800

nm, 1200 nm, and 1900 nm excitation wavelength. The verification values of power-dependent fluorescence intensity are 0.96 (800 nm), 1.85 (1200 nm), and 3.46 (1900 nm), indicating that $[\text{Fe}(\text{tpy})_2]^{2+}$ exhibits higher-order multiphoton behavior in the extended near-infrared range. Encouraged by the superior multiphoton absorption of $[\text{Fe}(\text{tpy})_2]^{2+}$, we further evaluate whether NHC coordination triggers MPEF. $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$ demonstrates activated three-photon excited fluorescence upon 1200-1500 nm wavelength excitation (Figure 1C). Benefiting from a strong ligand- field, $[\text{Fe}(\text{imp})_2]^{2+}$ exhibits a 17-fold enhancement in MPEF intensity (Figure 1D). It also displays a large three-photon absorption cross-section of $13.36 \times 10^{-78} \text{cm}^6 \text{s}^2 \text{photon}^{-2}$ along with a power-dependent emission slope of 3.39, which verifies excellent three-photon activity (Figure 1E).

The femtosecond transient absorption (fs-TA) spectra of $[\text{Fe}(\text{tpy})_2]^{2+}$ are dominated by a long-lived ground-state bleaching (GSB) feature, with its peak position corresponding to the UV-vis absorption spectra (Figure 1F and S13). For $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$, markedly accelerated GSB- signal decay indicates that NHC ligand perturbs the dominant MC- mediated deactivation pathway^[38] (Figure 1G). Upon further stronger NHC- ligand substitution, $[\text{Fe}(\text{imp})_2]^{2+}$ displays a distinct excited-state absorption (ESA) component above 525 nm, which decays almost synchronously with the GSB, thereby providing direct evidence for the formation of a photoactive charge-transfer excited state. Kinetic exponential fitting reveals that the decay kinetics of both $[\text{Fe}(\text{tpy})_2]^{2+}$ and $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$ consist of a short-lived component of about 100 fs and a nanosecond-scale long-lived component. The short-lived component originates from rapid ¹MLCT deactivation, while the nanosecond-scale component stems from a persistent non-radiative MC-state population responsible for the weak luminescence (Figure S14). In contrast, kinetic exponential fitting of $[\text{Fe}(\text{imp})_2]^{2+}$ yields a time constant of about 15 ps, corresponding to the ³MLCT lifetime (Figure 1I), corroborating the role of strong σ -donation in prolonging the charge-transfer lifetime.

This mechanistic difference is further supported by fluorescence lifetime measurements. $[\text{Fe}(\text{imp})_2]^{2+}$ features a far more accessible radiative decay channel, yielding nanosecond- scale lifetimes that greatly outperform $[\text{Fe}(\text{tpy})(\text{imp})]^{2+}$ (Figure 1J). However, given this pronounced discrepancy between the picosecond- scale ³MLCT lifetime and the nanosecond- scale emission lifetime, the luminescence of $[\text{Fe}(\text{imp})_2]^{2+}$ cannot be governed solely by direct MLCT emission and should arise from an additional emissive process. $[\text{Fe}(\text{imp})_2]^{2+}$ exhibits steadily increase emission intensity and a ~45 nm blueshift upon heating, typical signatures of TADF (Figure 1K)^[42-43]. Temperature-dependent lifetime measurements further substantiate this assignment (Figure 1L). The emission lifetime is progressively lengthened from approximately 1.46 ns to 1.69 ns as the temperature increases from 230 K to room temperature. Thermal activation promotes reverse population of an emissive charge-transfer state from a dark state, thereby facilitating delayed radiative decay.

On the basis of the above observations, NHC- ligand incorporation strengthens the ligand field and remodels the low-lying MC- state landscape, suppressing the typical ultrafast non- radiative decay channel prevalent in Fe complexes. Consequently, the system favors thermally activated repopulation of the emissive charge- transfer state, which undergoes radiative relaxation to the ground state via a TADF pathway (Figure 1M). These results prove that NHC-driven ligand-field strengthening is an efficient strategy to activate and boost MPEF in Fe-complexes. Notably, this tuning strategy endows $[\text{Fe}(\text{imp})_2]^{2+}$ with a usable nanosecond emission lifetime, breaking the limitation of ultrashort-lived Fe-based luminophores and revealing its promise as a multiphoton fluorescence lifetime probe.

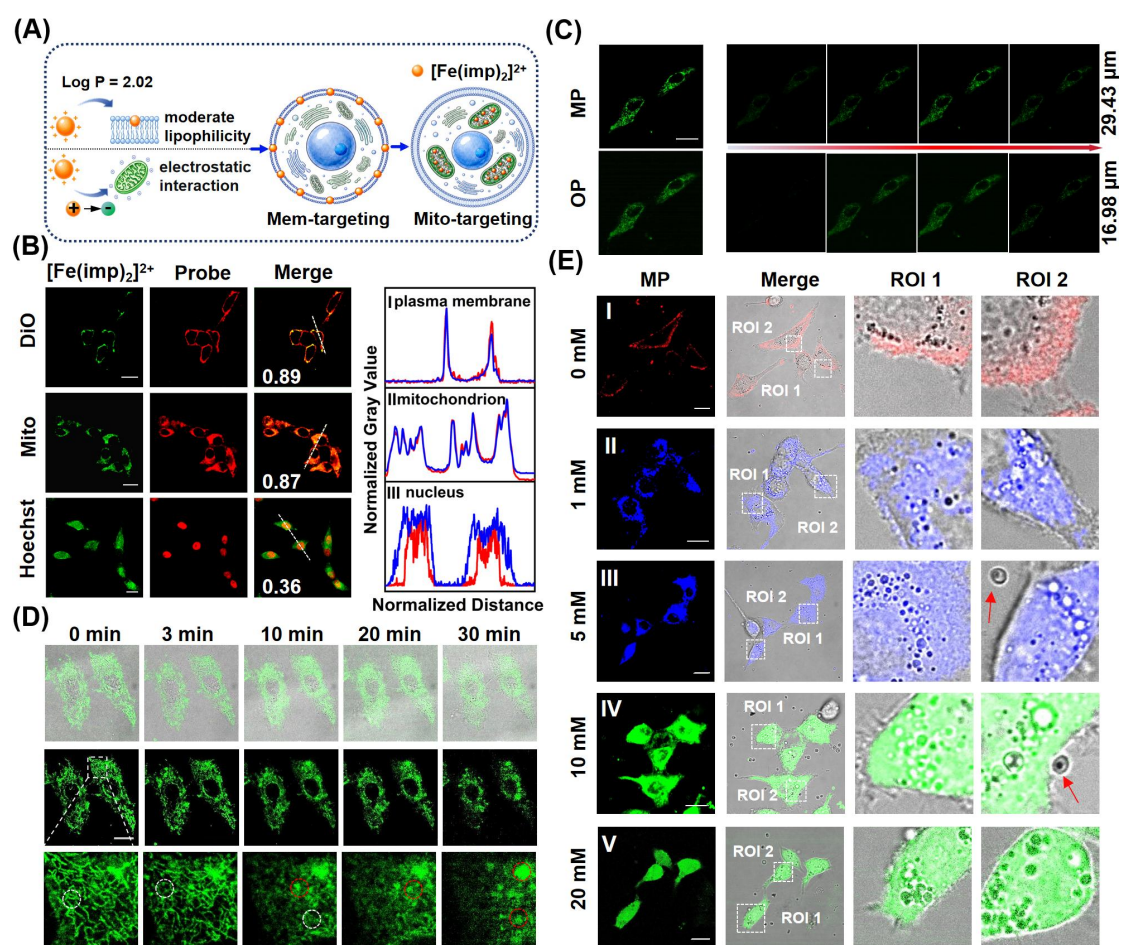


Figure 2. (A) Mechanisms of subcellular organelle translocation of $[\text{Fe}(\text{imp})_2]^{2+}$. (B) Laser confocal scanning imaging of different organelle targets and associated mean fluorescence intensity image in Hep G2 cells with $[\text{Fe}(\text{imp})_2]^{2+}$ and commercial dyes. (C) Penetration depth of $[\text{Fe}(\text{imp})_2]^{2+}$ in Hep G2 cells under confocal multi-photon and one-photon imaging. (D) Multi-photon fluorescence imaging tracks the fine structural changes of mitochondria from early to late apoptosis (White circle: filamentous mitochondria; red circle: fragmented swollen mitochondria). (E) Multi-photon fluorescence imaging of $[\text{Fe}(\text{imp})_2]^{2+}$ in cells at different state. Hep G2 cells are first treated with different concentration H_2O_2 (0, 1, 5, 10, 20 mM) for 12 h, followed by incubation with 10 μM $[\text{Fe}(\text{imp})_2]^{2+}$ for 30 min (Excitation wavelength: 800 nm; Emission wavelength: 500-650 nm; Scale bar: 20 μm).

Multiphoton Fluorescence Imaging for Dynamic Visualization of Apoptosis Progression

We next explore its performance as a multiphoton probe for live-cell apoptosis imaging. The subcellular

redistribution of $[\text{Fe}(\text{imp})_2]^{2+}$ stems from its amphiphilic cationic nature. Its butyl-functionalized NHC ligands offer moderate lipophilicity, and the positively charged Fe (II) core enables electrostatic binding to negatively charged subcellular organelles (Figure 2A, Figure S19). The probe signal strongly co-localizes with Dio fluorescence, pointing to predominant plasma-membrane localization in viable Hep G2 cells. $[\text{Fe}(\text{imp})_2]^{2+}$ internalizes and accumulates within mitochondria upon advancing cellular damage and increased membrane permeability. Progressive loss of cellular integrity drove further probe redistribution: first toward the nucleus, then throughout the cytoplasm. These observations clarify the inherent subcellular localization of $[\text{Fe}(\text{imp})_2]^{2+}$ in viable cells. This information lays a foundation for investigating the probe's translocation behavior during apoptosis (Figure 2B). Leveraging the excellent MPEF of $[\text{Fe}(\text{imp})_2]^{2+}$, 800 nm excitation is selected for all subsequent experiments as it provides a superior penetration depth of 29.43 μm , which is critical for high-resolution imaging. (Figure 2C)

Based on its specific mitochondrial accumulation in early apoptosis, we target this key organelle for dynamic morphological monitoring. The enrichment of $[\text{Fe}(\text{imp})_2]^{2+}$ in early apoptosis clearly reveals the extensive tubular mitochondrial network surrounding the nucleus. As apoptosis proceeds, this network undergoes progressive fragmentation, yielding short, rod-like, and spherical structures that ultimately swell into spherical fragments. This organelle-level morphological tracking is further extended to monitor the entire apoptotic cascade (Figure 2D). We next employ an H_2O_2 -gradient-induced apoptotic Hep G2 cell model to probe the dynamic targeting of $[\text{Fe}(\text{imp})_2]^{2+}$ during apoptosis (Figure 2E). $[\text{Fe}(\text{imp})_2]^{2+}$ primarily resides at the plasma membrane in viable cells with intact membrane architecture, where it shows relatively weak fluorescence (I). Upon early-stage apoptosis induction, elevated membrane permeability promotes $[\text{Fe}(\text{imp})_2]^{2+}$ internalization, enabling the probe to target and clearly delineate the filamentous mitochondrial network (II). Progressive apoptosis induces mitochondrial vesiculation, swelling, and membrane rupture, releasing $[\text{Fe}(\text{imp})_2]^{2+}$ into the cytosol (III and IV). The probe diffuses throughout the entire cell alongside a pronounced fluorescence enhancement, which is characterized by cellular swelling and loss of membrane integrity in late-stage apoptosis (Figure V). These results reveal that $[\text{Fe}(\text{imp})_2]^{2+}$ visualizes apoptotic progression in real time via sequential organelle redistribution, tracking dynamic intracellular remodeling rather than static apoptotic snapshots.

Multiphoton Fluorescence Lifetime Imaging for Quantitative Visualization of Apoptosis

In mid-to-late apoptotic cells, MP-FLIM imaging reveals pronounced spatial heterogeneity in fluorescence lifetimes (Figure 3A). This TADF-induced nanosecond lifetime provides quantitative micro-environmental information at the organelle level, beyond the dynamic localization offered by fluorescence imaging. Channel separation analysis shows that short-lifetime signals are mainly associated with mitochondrial regions, whereas long-lifetime signals are

distributed around the mitochondrial periphery and cytoplasmic region. This heterogeneous lifetime profile correlates with apoptosis-triggered mitochondrial permeabilization, swelling, elevated cytoplasmic viscosity^[44-45] and structural damage (Figure S20). Further investigation examines mitochondrial morphological changes during the mid-to-late stages of apoptosis. MP-FLIM imaging reveals the dynamic fragmentation of mitochondrial filaments, alongside a gradual decrease in lifetime differences between regions I and II, indicating declining lifetime heterogeneity (Figure 3B).

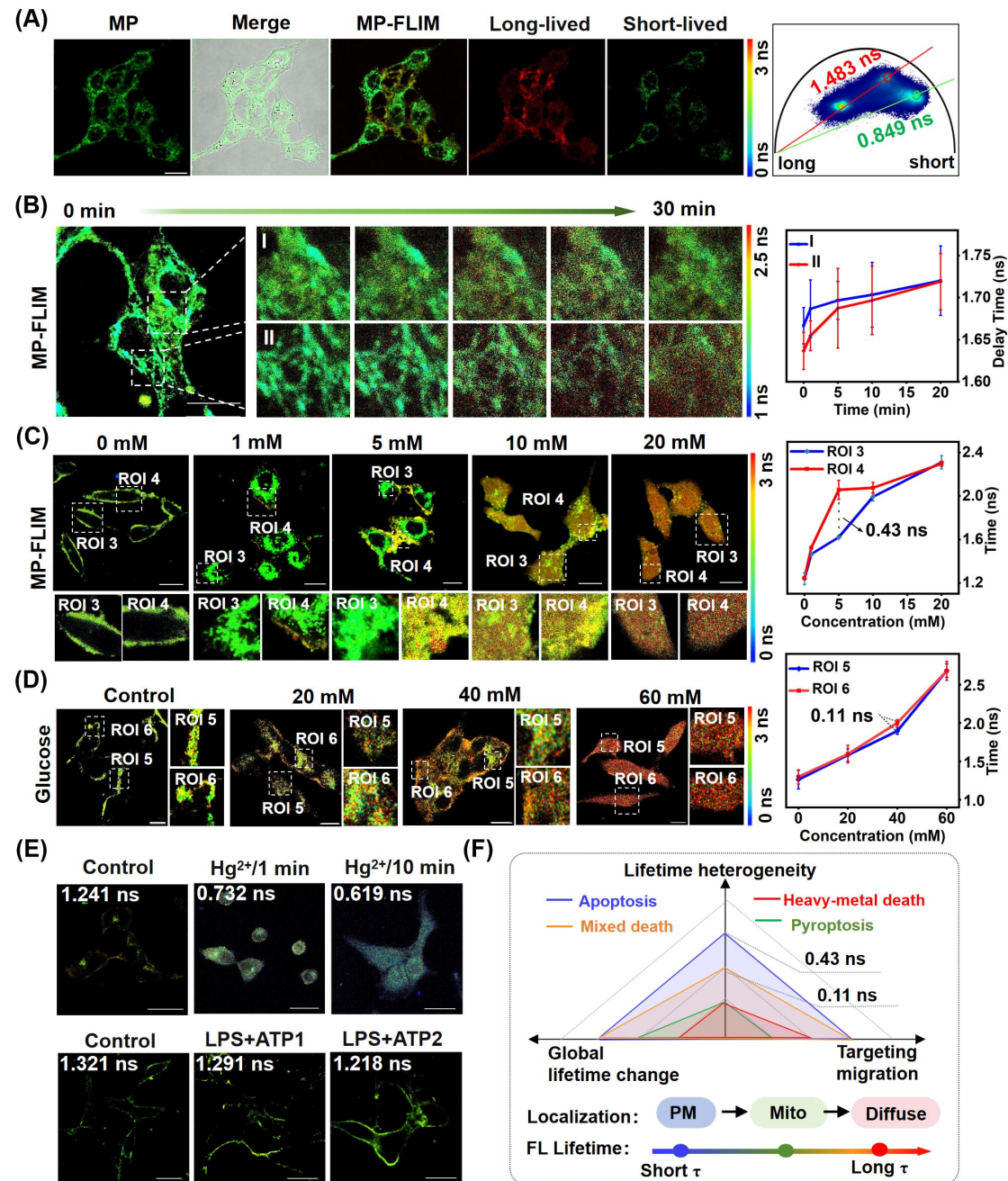


Figure 3. (A) Left: multi-photon fluorescence and MP-FLIM imaging of cells co-incubated with $[\text{Fe}(\text{imp})_2]^{2+}$ during mid-apoptotic state; Right: lifetime phasor plot analysis of $[\text{Fe}(\text{imp})_2]^{2+}$. (B) Left: time-dependent MP-FLIM imaging of cells in mid-apoptotic state after co-incubation with $[\text{Fe}(\text{imp})_2]^{2+}$; Right: Quantitative fluorescence lifetime maps of I and II. (C) Left: MP-FLIM imaging of $[\text{Fe}(\text{imp})_2]^{2+}$ in cells at different state; Right: quantitative fluorescence lifetime maps of ROI 3 and ROI 4. Hep G2 cells are first treated with different concentration H_2O_2 (0, 1, 5, 10, 20 mM) for 12 h, followed

by incubation with 10 μM $[\text{Fe}(\text{imp})_2]^{2+}$ for 30 min. (D) Left: MP-FLIM imaging of Hep G2 cells co-incubated with different glucose concentrations (0, 20, 40, 60 mM) and then with $[\text{Fe}(\text{imp})_2]^{2+}$; Right: quantitative fluorescence lifetime maps of ROI 5 and ROI 6. (E) MP-FLIM imaging of Hg^{2+} -induced heavy metal poisoning models and pyroptosis models. (F) Three-dimensional parameter comparison analysis chart for distinguishing different causes of death. (Excitation wavelength: 800 nm; Emission wavelength: 500-650 nm; Scale bar: 20 μm).

Given the high lifetime sensitivity of $[\text{Fe}(\text{imp})_2]^{2+}$ to organelle status, we employ it as a quantitative molecular platform to convert apoptotic morphological dynamics into measurable lifetime signals. $[\text{Fe}(\text{imp})_2]^{2+}$ exhibits an initial plasma membrane localization and a short 1.24 ns lifetime, while FLIM reveals its apoptotic translocation to mitochondria along with a significant lifetime enhancement. It displays distinct lifetime heterogeneity with a measurable 0.43 ns lifetime disparity, upon mitochondrial structural breakdown and diminished probe organelle specificity (Figure 3C). In the late stages of apoptosis, Hep G2 cells display a red-covered long-lifetime channel, with the lifetime increasing to 2.30 ns, representing a net increase of 1.06 ns compared to the initial state. To verify whether such lifetime heterogeneity is apoptosis-specific, we further examine alternative cell death models and observe pronounced global fluorescence lifetime elevation from 1.28 ns to 2.64 ns under glucose-triggered mixed cell death. However, lifetime heterogeneity remains minimal (0.11 ns) due to glucose-activated complicated cell death cascades (Figure 3D). The probe's lifetime drops sharply and diffuses uniformly across the cytoplasm from the plasma membrane under Hg^{2+} heavy metal stress. In pyroptosis, the probe persistently localizes to membrane with negligible lifetime shifts (Figure 3E). We thus built a multidimensional parameter map using the TADF-based MP-FLIM approach to accurately detect apoptosis. This map combines three complementary metrics, including organelle translocation, globally prolonged fluorescence lifetime, and sub-hundred-picosecond lifetime heterogeneity, thereby distinguishing apoptosis from other death pathways (Figure 3F).

Quantitative assessment of anti-apoptotic efficacy by dual-parameter MP-FLIM

Taking advantage of the multiphoton- excitable and lifetime- responsive features of the $[\text{Fe}(\text{imp})_2]^{2+}$, we then applied it to track probe localization and lifetime changes for assessing the protective effects of anti- apoptotic agents. Hep G2 cells are pretreated with L-ascorbic acid or quercetin at different concentrations, followed by H_2O_2 -induced apoptosis and subsequent incubation with $[\text{Fe}(\text{imp})_2]^{2+}$ for MP fluorescence and MP-FLIM imaging (Figure 4A). Low-dose L-ascorbic acid fails to suppress H_2O_2 -induced apoptosis. This is confirmed by partial redistribution of $[\text{Fe}(\text{imp})_2]^{2+}$ from mitochondria to the nucleus and persistently prolonged luminescence lifetimes. Elevating L-ascorbic acid concentrations restores normal mitochondrial morphology. $[\text{Fe}(\text{imp})_2]^{2+}$ accumulates selectively within mitochondria, accompanied by a reduction in overall luminescence lifetime. Maximal protective effects are observed with 150 μM L-ascorbic acid, at which both fluorescence distribution and lifetime signals recover toward the normal cellular state

(Figure 4B ,4D and 4E). A similar concentration-dependent trend is observed for quercetin (Figure 4C, 4F, and 4G). The optimal anti-apoptotic effect is observed at 50 μM , at which the apoptosis-induced probe redistribution and lifetime shifts are effectively counteracted. Overall, these findings demonstrate that the combination of fluorescence intensity and lifetime parameters enables reliable evaluation of anti-apoptotic potency, establishing $[\text{Fe}(\text{imp})_2]^{2+}$ as a promising MP-FLIM probe for apoptosis-related drug screening (Figure 4H).

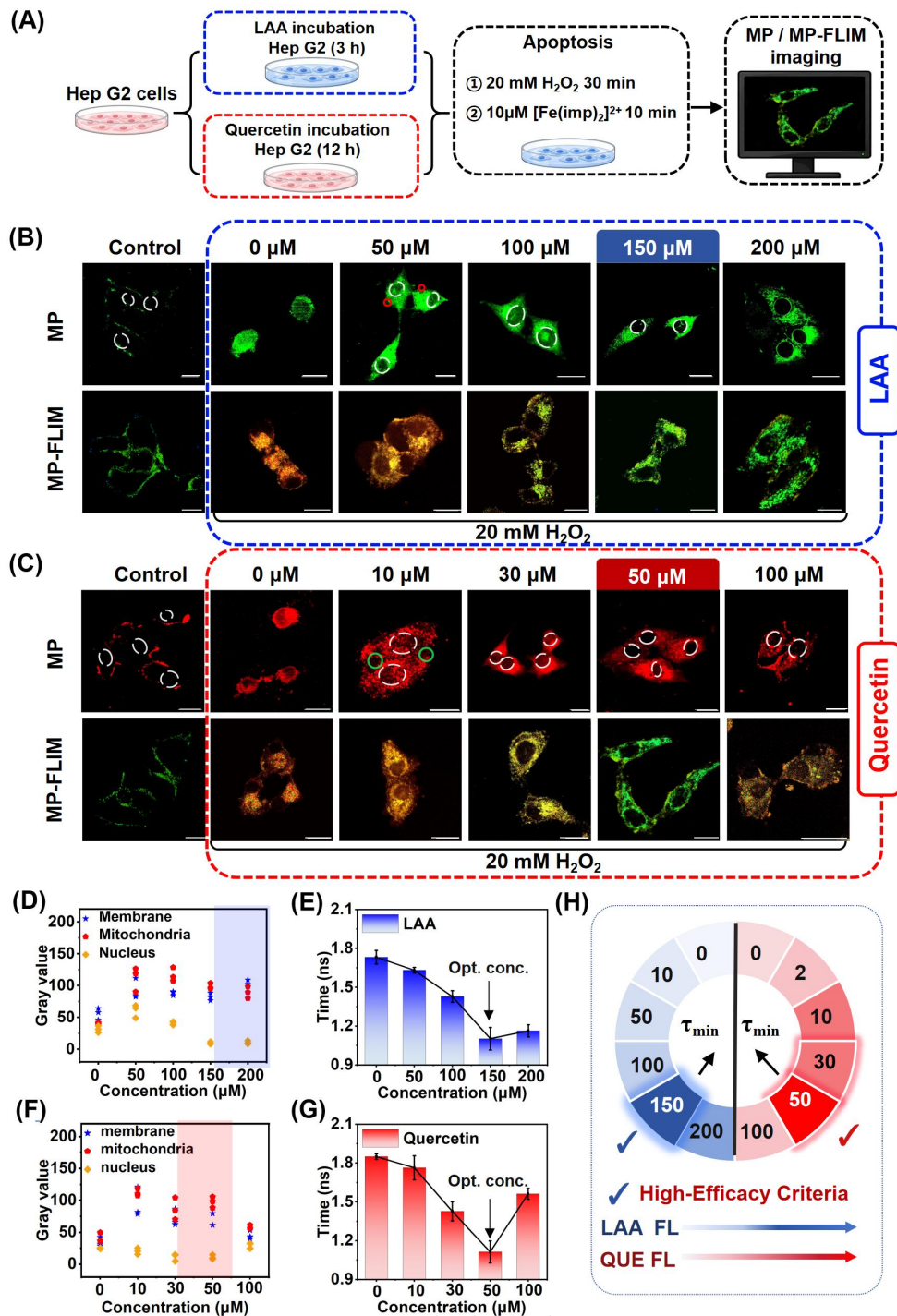


Figure 4. (A) Flowchart of $[\text{Fe}(\text{imp})_2]^{2+}$ for evaluating the pharmacological efficacy of L-ascorbic acid and quercetin. (B) Multi-photon fluorescence confocal imaging and fluorescence lifetime imaging of cells pretreated with various

concentrations of L-ascorbic acid (0, 50, 100, 150 200 μM), followed by H_2O_2 -induced apoptosis, and then co-incubated with $[\text{Fe}(\text{imp})_2]^{2+}$ (10 μM). (C) Multi-photon fluorescence confocal imaging and fluorescence lifetime imaging of cells pretreated with various concentrations of quercetin (0, 10, 30, 50, 100 μM), followed by H_2O_2 -induced apoptosis, and then co-incubated with $[\text{Fe}(\text{imp})_2]^{2+}$ (10 μM). (D) Statistical analysis of fluorescence intensities in three selected regions from different organelles shown in Figure B. (E) Corresponding fluorescence lifetime values measured in Figure B. (F) Statistical analysis of fluorescence intensities in three selected regions from different organelles shown in Figure C. (G) Corresponding fluorescence lifetime values measured in Figure C. (H) Dual-parameter calibration for optimal drug concentration selection.

■ CONCLUSION

To sum up, this work innovatively reports a NHC regulated luminescent Fe (II) complex which is employed to dynamically track apoptosis-associated evolution. $[\text{Fe}(\text{imp})_2]^{2+}$ exhibits unprecedented 3PEF and TADF properties, effectively overcoming the intrinsically short-lived excited states inherent to Fe (II) systems. Taking advantage of the multiphoton-excitation and nanosecond-scale lifetime, $[\text{Fe}(\text{imp})_2]^{2+}$ facilitates dynamic visualization of apoptosis-associated organelle redistribution and fluorescence lifetime heterogeneity. Application-wise, $[\text{Fe}(\text{imp})_2]^{2+}$ provides a quantitative MP-FLIM platform for distinguishing apoptosis from other cell death pathways. In the presence of anti-apoptotic agents, optimal drug effects are achieved when $[\text{Fe}(\text{imp})_2]^{2+}$ is exclusively localized to the mitochondria, accompanied by progressive fluorescence lifetime attenuation. The long-lived multiphoton luminescence of Fe-based complexes provides a powerful foundation for quantitatively resolving dynamic and heterogeneous biological processes.

Data Availability

The data that support the findings of this study are available within the article and the supplementary information. Details about materials and methods, experimental procedures, characterizations and NMR spectra are available in the supplementary information. Source data are provided with this paper.

Author contributions

Yaqin Li designed the overall experimental scheme, conducted the tests, summarized data, and wrote the manuscript. Xiaowei Gong conducted part of the experimental tests and performed the data analysis. Qiong Zhang, Xuegang Chen and Hongping Zhou provided guidance on the overall experimental design and supervised the writing and revision of the manuscript. Zhou Lu and Yaxiong Wei supervised the transient absorption spectroscopy measurements and data analysis.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (22475002, 22473002, 52372073), The Excellent Scientific Research and Innovation Team of Anhui Province (2025AHGXZK10013), The Key Laboratory of Structure and Functional Regulation of Hybrid Materials of Ministry of Education, Anhui University (No.

JF202512010001 and 109/KZ32025033), AnHui Province Key Laboratory of Chemistry for Inorganic/Organic Hybrid Functionalized Materials, Anhui University (IOHFMAH-2501), Tongxin Science and Technology Innovation Project of Anhui Provincial Department of Science and Technology (202423a11020004). the Natural Science Foundation Project of Anhui Province (2408085MB048), the University Annual Scientific Research Plan of Anhui Province (2022AH010013), the 111 Project (D26004), the Key Project of State Key Laboratory of Opto-Electronic Information Acquisition and Protection Technology of Anhui University (OEIAPT20250201), Support Program for Outstanding Young Talents in Universities of Anhui Province (YQZD2025001).

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