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Mini Review

Recent Advances in FRET Technology for Medical Applications

Ling Jiang, Ping Li, Ying Jiang, Zhitao Hou, Tianzhu Guo, Ping Liu, Yuting Ji, Yan Qi, XinYue Zhang, Yuan Tian, XiaoxiaoQi, Ruonan Zheng and Yun Bai*

School Basic Medical Science, Heilongjiang University of Chinese Medicine, Heilongjiang University of Chinese Medicine, P.R. China

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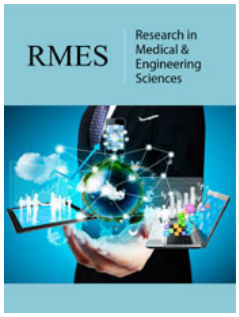

***Corresponding authors:** Yun Bai, School Basic Medical Science, Heilongjiang University of Chinese Medicine, Heilongjiang University of Chinese Medicine, 24 Heping Road, Harbin, 150040, P.R. China

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Abstract

Förster resonance energy transfer (FRET) has emerged as a pivotal biophysical tool in medical research, enabling high-sensitivity detection of molecular interactions and dynamic processes. Recent technological advancements have significantly expanded FRET applications in cell biology and drug discovery, driving profound insights into disease mechanisms and therapeutic innovation. Despite its transformative potential, FRET implementation faces persistent challenges including signal intensity limitations, background noise interference, and experimental design constraints. This review synthesizes cutting-edge developments in FRET technology, critically evaluates its current medical applications, addresses key methodological challenges, and outlines future research trajectories to advance biomedical investigation.

Keywords: FRET; Biomedicine; Bioimaging; Cell biology; Drug discovery

Introduction

FRET technology leverages distance-dependent energy transfer between fluorescent molecules (donor and acceptor) to detect biomolecular interactions with nanometer-scale spatial precision (<10nm). This technique has become indispensable for monitoring real-time cellular signaling, elucidating disease pathways, and facilitating high-throughput drug screening. Recent innovations have broadened FRET's utility across diverse contexts from mapping single-molecule dynamics to profiling network-level interactions in complex biological systems [1]. Notable progress includes FRET-based monitoring of intracellular signaling cascades that reveal adaptive cellular responses to stimuli [2], identification of pathogenic protein interactions in cancer and neurodegenerative disorders [3], and accelerated drug candidate evaluation through multiplexed screening platforms [4]. This review examines fundamental principles and emerging applications of FRET technology across medical domains, highlighting its transformative potential in addressing critical biomedical challenges.

Fundamental principles and biomedical significance of FRET

A. Mechanism and Requirements FRET efficiency (E) follows Förster's equation $E = 1/[1+(R/R_0)^6]$, where R represents donor-acceptor distance and R_0 denotes the characteristic distance at 50% energy transfer. Optimal FRET occurs when spectral overlap exceeds 30%, dipole orientations are favorable, and interfluorophore distance remains within 1-10nm. These characteristics position FRET as a powerful "molecular ruler" for quantifying biomolecular interactions[1].

B. Advantages in Biomedical Research FRET provides unparalleled capabilities for live-cell dynamic monitoring with millisecond temporal resolution and single-molecule

sensitivity. Its compatibility with fluorescence lifetime imaging microscopy (FLIM) enables background-free quantification [5,6]. Recent advances including time-resolved detection and multiphoton excitation have further enhanced signal-to-noise ratios (>500:1) and tissue penetration depth (up to 800μm) [7].

FRET in cell biology

Dynamic monitoring of cellular signaling: Integration of FRET with two-photon fluorescence lifetime imaging microscopy (2P-FLIM) enables spatiotemporal mapping of kinase activation and second-messenger dynamics in thick tissues [7]. For instance, Rac1 GTPase activity gradients in migrating cells have been quantified using EGFP-mCherry FRET biosensors with subcellular resolution (±0.1 FRET efficiency units) [8-20].

Protein interaction analysis: Dual-labeling strategies with fluorescent proteins (e.g., CFP-YFP) reveal protein complex stoichiometry and dissociation constants *in vivo*. This approach has identified allosteric changes in β2-adrenergic receptors within 500ms of agonist stimulation.

Molecular diagnostics innovation: smFRET, a Single-molecule resolution achieves 10^{-18}M detection limits for nucleic acid dynamics and CRISPR-Cas activity monitoring [21,22]. TR-FRET, Europium chelate donors with time-gated detection reduce autofluorescence interference by 30-fold for cytokine storm diagnostics [23]. Quantum Dot (QD)-FRET, Water-soluble CdTe/ZnS core-shell QDs functionalized with 3-mercaptopropionic acid exhibit quantum yields >80% for quantitative metabolite sensing [24].

FRET in drug discovery

A. Target Validation TR-FRET binding assays screen interactions at physiological concentrations, outperforming ELISA in precision and throughput [25].

B. Screening and Mechanism Profiling Advanced FRET biosensors enable real-time drug activity assessment: Genetically encoded mNeonGreen-HaloTag systems track receptor dimerization kinetics Carbon nanotube-QD hybrids detect serum PD-L1 at 0.1pM in 5 minutes Lanthanide nanoparticle probes guide neurosurgical interventions with <50μm precision [26,27]. Integrated genotyping (e.g., LightSNiP + TR-FRET) identifies DPYD 6 variants predictive of fluoropyrimidine toxicity [28].

Conclusion

FRET is undergoing rapid development in medical applications, extending its influence to critical domains including cell biology and drug discovery. Through non-invasive means, FRET enables real-time monitoring of molecular interactions and dynamic processes within cells, significantly advancing our understanding of biological mechanisms. However, as FRET gains widespread adoption, researchers face challenges in balancing divergent perspectives. On the one hand, studies demonstrate FRET's superiority in monitoring molecular interactions and dynamic changes, providing high-resolution, real-time intracellular information. On the other hand, concerns exist regarding its accuracy in complex biological systems, where signal interference and background noise may compromise result reliability. To reconcile these views, future research must prioritize standardization and optimization of FRET technology for precise application across diverse biological environments.

Continual advancements in FRET bring both challenges and opportunities. Innovations in probe development, imaging technology, and data analysis methods are expanding FRET's potential. These improvements will enhance its value in basic research and accelerate clinical translation for early disease diagnosis and personalized therapies.

Future studies should persistently explore novel FRET applications and integrate it with complementary bioimaging technologies to further medical research. Through interdisciplinary collaboration, researchers can achieve comprehensive understanding of complex biological systems, providing robust support for clinical practice. In summary, while FRET faces significant challenges, its promising future in medicine holds undeniable potential to profoundly impact biomedical research.

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Conflicts of interest

There are no conflicts to declare.

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