Research on the Application of Gene Coding Fluorescent Probes

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Abstract. Genetically encoded fluorescent probes, renowned for their biocompatibility and suitability for live-cell detection, have emerged as a pivotal tool in the field of molecular and ionic detection. These probes are characterized by their high specificity, exceptional editability, and rapid imaging capabilities, making them indispensable in many biological assays. This review aims to elucidate the mechanisms and applications of gene-encoded fluorescent probes in metal ion detection, neurotransmitter imaging, and cell metabolism detection. Specifically, probes constructed through gene programming can cause changes in fluorescence intensity by binding specifically to target substances, thereby achieving accurate concentration detection and real-time imaging of target substances. The biological compatibility of gene-encoded fluorescent probes is good, and they can be used in living cells, so they also have important value in the detection of physiological and pathological indicators. This review will also delineate the comparative advantages of these probes over traditional detection methods and offer insights into the prospective trajectory of this burgeoning technology.

Keywords: Genetically encoded fluorescent probe, metal ion detection, neurotransmitter imaging, cellular metabolism.

1. Introduction

Genetically encoded fluorescent probes are tools that utilize genetically encoded proteins or nucleic acid molecules to label and visualize specific target substances within cellular environments. The capacity of these probes for real-time localization, dynamic imaging, and quantitative detection of substances in living cells underscores their significant application value in the biomedical field, particularly in biological metabolism research, neuroscience, and early disease screening. Currently, a variety of genetically encoded fluorescent probes targeting diverse substances (such as ions, biological signaling molecules, and cellular metabolites) have been developed [1-3], exhibiting high specificity and sensitivity alongside excellent biocompatibility and low toxicity; they also possess reusability and programmable control features. Furthermore, the advancement of multi-color probes facilitates simultaneous monitoring of multiple biomolecules within a single sample, thereby greatly enhancing detection efficiency.

However, the clinical application of genetically encoded fluorescent probes remains limited. Despite notable progress in this technology's research domain, improvements in sensitivity and resolution are still necessary—particularly concerning deep tissue imaging—which presents challenges related to fluorescence penetration and stability. Additionally, since these probes fundamentally consist of proteins or nucleic acids, their structural integrity can be compromised under unsuitable temperature or pH conditions; thus, there is a need for the development of probes tailored for various complex environments. Conversely, the full potential advantages offered by genetically encoded fluorescent probes have yet to be realized due to constraints imposed by advancements in artificial protein synthesis and nucleic acid technologies. Presently available probes predominantly target common biomolecules or ions; those aimed at special structures or rarer substances remain largely experimental. There is also considerable scope for enhancement regarding both specificity and detection efficiency among existing probe designs. With emerging technologies coupled with enhancements to current methodologies, it is anticipated that these gaps will gradually close.

This article primarily discusses the principles underlying genetically encoded fluorescent probe technology along with its applications in detecting various metal ions as well as neurotransmitter imaging—and specifically focuses on three metabolites: APT (adenosine triphosphate), NADPH (nicotinamide adenine dinucleotide phosphate), and lactate—to promote further advancements within this field.

2. Principle

The components of genetically encoded fluorescent probes comprise recognition elements and signal-generating elements for fluorescence. The former consists of nucleic acid segments with specific sequences or proteins endowed with recognition capabilities that can selectively bind to target analytes; the latter refers to a protein capable of producing stable fluorescence. Upon the binding of the recognition element to the target substance, alterations in activity, spectral properties, signal intensity, and other characteristics of the fluorescent protein occur by the concentration of the target substance, thereby enabling both qualitative and quantitative measurement of analyte concentration [4].

Currently, functional nucleic acids that have been widely applied can be divided into aptamers which have recognition functions similar to antibodies, deoxy ribozymes which have catalytic functions similar to protein enzymes, and molecular beacons. These types of nucleic acid probes have the advantages of simple structure, high specificity, and high sensitivity, making them suitable for the detection of concentrations of various metal ions, neurotransmitters, and cellular metabolic substances [5].

3. Applications

3.1. Metal ion Detection

Metal ions play a crucial role in biological activities. For example, iron is the most abundant transition metal element in the human body, participating in cellular composition and the transport and storage of oxygen. The ability of its different valence ions to interconvert and its strong redox capacity are essential for oxygen storage and transport within the body. However, the strong redox capacity of iron can also generate free radicals that damage cellular structures, conferring potential cytotoxicity. In addition, the abnormal accumulation of iron in brain tissue can lead to neurodegenerative diseases, such as Alzheimer's disease [6]; When cells undergo inflammation, carcinogenesis, or aging, it may lead to the loss of iron homeostasis [7-9]. Therefore, detecting the concentration of metal ions has physiological and pathological significance.

Traditional methodologies for metal ion detection include electrochemical detection, optical detection, and biological detection, which suffer from drawbacks such as high cost, bulky equipment, complex operation, and slow analytical speeds [10]. In contrast, genetically encoded fluorescent probes offer several advantages, including better cost-effectiveness, high precision, facile synthesis, reusability, and programmable control. These probes leverage the specificity of molecular recognition elements and the sensitivity of fluorescent signal generators, providing a versatile platform for the detection and quantification of metal ions in biological systems.

Potassium, the most abundant cation in the cell, can coordinate with guanine and form structurally stable aptamers with G4 rich in guanine. G4 has a diverse topological structure, and based on this principle, potassium ion detection can be achieved in the presence of sodium ions, by designing highly selective potassium ion probes [11]. Previous studies have shown that zinc ions, magnesium ions, and ferrous ions all exhibit the potential for specific cleavage of deoxy ribozymes or RNA [12, 13], which can be applied for sensing and imaging these metal ions. Many heavy metal ions, such as lead and mercury, readily form stable compounds with nucleic acid molecules among them, lead ions have a high selectivity for G5R, and in 1994, a DNA lead ion probe based on G5R was designed [14]. The T-Hg^{2+}-T mismatched base formed by the specific interaction between thymine and mercury ions

can be used to construct molecular beacon sensors [15]. The binding with mercury ions can make the mismatched base form a hairpin structure, thus enhancing the fluorescence resonance energy transfer and inducing the fluorescence of the receptor molecule. In 2023, Zhenying Chen et al. constructed a novel fluorescent probe for silver ions based on Pepper (a fluorescent RNA without RG4 motif), showing high selectivity, high-resolution chain temperature and wide pH tolerance, which can be applied to real-time detection of silver ions in living cells. The principle is that in the absence of silver ions, base pair mismatches result in low fluorescence, while in the presence of silver ions, C-Ag^{+}-C metal base pairs are formed to stabilize the stem-loop structure, achieving a 3.7-fold fluorescence enhancement. Moreover, compared with other fluorescent RNA aptamers, this probe not only has the advantages of traditional genetically encoded probes but also has an affinity that can be enhanced by one to two orders of magnitude [16].

In summary, the principle of metal ion imaging of gene-encoded fluorescent probes can be summarized as the specific cutting of nucleic acids by metal ions, or the binding of metal ions and nucleic acids into stable structures to stimulate fluorescence. Due to the universality of its principle, the same method can be used to develop detection tools for different metal ions by reprogramming the probe. Therefore, gene-coded fluorescent probes can be widely used in metal ion detection.

3.2. Neurotransmitter Imaging

Neurotransmitters can be primarily categorized into cholinergic, monoaminergic, amino acidergic, and neuropeptidergic classes. They are chemical substances used for intercellular signaling within the nervous system and play a crucial role in the synaptic connections between neurons, mediating the transmission of information. Neurotransmitters are not only involved in numerous physiological processes such as movement, memory, and perception, but their abnormal release can also lead to a variety of neurological disorders. For example, one of the characteristic pathological changes of Parkinson's disease is the progressive loss of dopamine neurons; neurotransmitter dysregulation is closely associated with the etiology and symptoms of autism spectrum disorders; Moreover, neurotransmitter dysfunction may also be related to the pathological mechanisms of bipolar disorder and depression.

Neurotransmitter imaging methods mainly include microdialysis, electrochemical methods, and fluorescence imaging. The secretion mechanisms of neurotransmitters are complex and difficult to detect, with traditional methods suffering from low specificity and the need for improved spatiotemporal resolution. Genetically encoded fluorescent probes have shown significant potential for optimization and are expected to provide more effective means for detecting neurotransmitters.

Probes for detecting neurotransmitters can be divided into those based on periplasmic binding proteins (PBPs) and those based on G protein-coupled receptors (GPCRs). PBPs are located extracellularly, and this type of probe is suitable for detecting small molecules outside the cell membrane, such as glutamate, acetylcholine, serotonin, etc. The PBP-based sensor GluSnFR fuses a fluorescent protein with a glutamate-binding protein, like the bacterial periplasmic glutamate/glutamine-binding protein gltI, to construct a probe capable of detecting dynamic changes in neuronal glutamate. At present, researchers have developed an optimized variant iGluSnFR3.v857 [17], which has higher sensitivity (approximately 20 times more responsive than SF-iGluSnFR) and superior kinetic properties (twice as fast as the previous generation SF-iGluSnFR), allowing for more accurate monitoring of glutamate signals both synaptically and extrasynaptically. In contrast, GPCRs are transmembrane proteins, and probes designed based on them have good affinity and extremely high sensitivity for endogenous neurotransmitters within cells, making them suitable for larger neurotransmitter molecules such as neuropeptides [18, 19]. In 2018, Marvin et al. developed a GPCR-based variant of iGluSnFR with brighter fluorescence intensity, achieving detection accuracy of millimoles and capable of producing different colored fluorescence at a rate of kilohertz [20].

The advantages of genetically encoded fluorescent probes lie in their ability to directly, rapidly, and with high specificity detect trace amounts of neurotransmitters in vivo. In 2018, the GPCR-based genetically encoded dopamine sensor $GRAB_{DA}$ was successfully applied for the direct measurement

of dopamine in fruit flies, fish, and mice. This probe can exhibit a 90% fluorescence amplification, with sub-second kinetics, nanomolar to sub-nanomolar affinity, and extremely high molecular specificity [21]. Because of these properties, genetically encoded probes can be used to accurately distinguish and detect different neurotransmitter molecules within the same brain region, which is helpful for disease screening and diagnosis. The development of new protein variants in the future may lead to probe designs with higher sensitivity, stronger, and more stable fluorescence increases, thereby improving diagnostic efficiency, deepening the understanding of the mechanisms behind neurotransmitter dysregulation or neurodegenerative diseases, and providing new avenues for disease treatment.

3.3. Cell Metabolism Detection

Cellular metabolism is fundamental to sustaining life, and metabolic dysregulation may cause diseases such as diabetes, hyperlipidemia, hyperuricemia, etc. Detecting cellular metabolic levels can effectively prevent these corresponding diseases, and genetically encoded fluorescent probes have demonstrated advantages in this field, such as the ability to perform in vivo imaging, dynamic imaging, high precision, and rapid speed.

ATP is a direct source of energy for human cells, and its abnormal metabolism is associated with diseases such as malignant tumors. To detect ATP, Yulong Li and his team developed an ATP sensor based on human ATP receptor P2Y1 and cpEGFP, called GRAB ATP1.0, which has high molecular specificity, sub-second-level kinetics, and nanomolar affinity for ATP, and has strong fluorescence response both inside and outside the body. Furthermore, this technology has been successfully applied to detect the characteristics of ATP release during local tissue damage in zebrafish and the extracellular ATP signals in the brains of mice during immune responses [22].

NADPH is involved in the regulation of anabolic reactions and redox balance. However, due to its complex structure, there are still challenges in detecting this substance. In 2024, Lu Wang and his team developed a protein-responsive fluorescent probe for NADPH detection, namely the FOCS-NADPH probe. Compared to conventional probes, this probe has high specificity and signal-to-noise ratio, is less susceptible to the influence of other metabolic molecules with similar structures and environmental pH, and responds quickly and reversibly. The fluorescence signal enhancement caused by the binding of the probe to the target molecule can reach hundreds of times, which can achieve wash-free imaging of target proteins in living cells [23].

Lactic acid is produced through glucose oxidation during energy metabolism and serves as a metabolic indicator of disease states. Since the production and degradation of lactic acid are dynamic processes, the detection of lactic acid requires extremely high spatiotemporal resolution and sensitivity. The lactate sensor FiLa developed by Li Xie et al. is characterized by high sensitivity and rapid response, enabling real-time monitoring of lactic acid metabolism in living cells. After binding with lactic acid, the probe can produce a 1500% fluorescence ratio change, with a dynamic range of about 470% to 2600%, and a lactic acid affinity range of ∼19μM to ∼1mM. Moreover, the temperature and pH inside the organism have little effect on the probe, so the FiLa sensor can be used for lactate detection in vitro and in vivo [24].

The future development directions of genetically encoded fluorescent probes in metabolic detection may include enhancing the luminescent efficiency of the probes to meet the demands for higher imaging speeds and deeper tissue imaging, improving the functional modification characteristics of the probes to enhance specificity and compatibility, and developing multi-color probes for metabolic analysis in different pathways and regions. These technologies make it possible to dynamically observe biological metabolism in real-time.

4. Conclusion

Metal ions are integral to numerous biological processes. Traditional metal ion detection methods have various limitations, whereas genetically encoded fluorescent probes offer advantages such as

low cost, high specificity, and programmable control capabilities, thereby demonstrating substantial potential for application in metal ion detection. These probes achieve concentration detection and imaging of target ions by specifically cleaving nucleic acids or binding to base pairs to excite fluorescence. Given the shared principles underlying these technologies, it is anticipated that additional fluorescent probes targeting diverse metal ions can be developed via reprogramming strategies, thus advancing related biomedical research and clinical diagnostics.

Neurotransmitters are involved in various physiological processes, with their dysregulation linked to several neurological disorders. Compared with traditional detection methods, genetically encoded fluorescent probes provide a detection method that is biocompatible, fast and has high sensitivity and specificity. These probes can be categorized into two primary classes: periplasmic binding protein (PBP)-based sensors suitable for detecting small extracellular neurotransmitters and G protein-coupled receptor (GPCR)-based sensors designed for larger intracellular neurotransmitter detection. This technology can accurately detect and distinguish different neurotransmitter concentrations in the same brain region in real-time, which helps deepen the understanding of the mechanisms of neurotransmitter dysfunction and provides novel avenues for diagnosing and treating associated diseases.

Dysregulation of cellular metabolism can precipitate a range of diseases; genetically encoded fluorescent probes possess notable advantages such as high affinity and sensitivity when measuring cellular metabolic levels. By binding specifically to metabolic substrates while exciting fluorescence signals, these probes capture subtle fluctuations in metabolite concentrations within living cells. Their high-precision detection helps to further promote the development of metabolic research and disease diagnosis.

The demand for the application of gene encoded fluorescent probes is constantly increasing in fields such as neuroscience, tumor diagnosis, and gene screening. However, there are still unresolved issues with this technology. For example, in complex environments, the accuracy of gene encoded fluorescent probes may be affected by pH and temperature; additionally, improvements are needed regarding response time stability intensity penetration during live cell imaging scenarios; furthermore interference from structurally similar substances may skew concentration measurements obtained via probing techniques. In the future, this technology may undergo cross disciplinary integration with other disciplines. For example, the development of synthetic biology has provided the possibility for the artificial synthesis of new high-performance fluorescent proteins, which will open up new paths for the design and application of gene encoded fluorescent probes, enabling them to develop in a wider range of applications, higher sensitivity and specificity, faster response time, stronger fluorescence amplification and stability, and other directions; multimodal imaging combined with image post-processing methodologies integrated alongside artificial intelligence could enable more accurate analyses concerning target substance concentrations across varied temporal-spatial contexts ultimately positioning this technology at the forefront of early disease screening initiatives while offering valuable insights guiding diagnostic practices.

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