Molecular interaction-based fluorescence sensing technologies and their application

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Abstract. Sensing technology is a cutting-edge technological approach, which makes it much easier to extract information. Molecular interaction is an indispensable part in the sensing technology process, where the chemical, optical and electrical properties of the system change, and it also plays an important role in the information processing and sample detection. However, there are various problems with most of the current sensing technologies, such as low sensitivity, low selectivity, complicated operation, so they cannot be universally used in applications in various fields. By contrast, the high sensitivity of fluorescence detection technology enables the analytes in species could be detected at very low levels. More importantly, it can provide information on the temporal and spatial distribution of the analyzed species without damaging biological samples, which is of great value for studying the physiological functions of these species. This paper introduces three kinds of molecular interaction-based fluorescence sensing technologies and elaborates their principles, applications in recent years, analyzing their merits and demerits respectively, and finally, concluding predecessors' research and prospecting the future trends of fluorescence sensing technologies. This paper lays the foundation of molecular interaction-based fluorescence sensing technologies and can provide background knowledge, which will offer help to the future research.

Keywords: molecular interaction; fluorescent probe; molecular orbital theory; ion detection; disease diagnosis.

1. Introduction

As an adcanced technical method, sensing technology makes the extraction of information much more convenient. The sensor responds to the analyte and converts the value into readable information. Among all the sensing technologies, the fluorescent probe method has the advantages of high sensitivity, high selectivity and simple operation. Most of the fluorescent probes are based on molecular interaction. The subjects and objects are combined with each other through the synergistic effect of intermolecular forces under specific conditions. During the detecting process, the chemical and optical properties of the system always change and can be detected by the probe. With the continuous deepening of research on fluorescent probes, scientists have developed a large amount of molecular interaction-based fluorescent probes, including Photoinduced Electron Transfer (PET), Intramolecular Charge Transfer (ICT), Fluorescence Resonance Energy Transfer (FRET), and so on. Those probes all have relatively complicated mechanisms, and due to excellent detecting performance, they have been widely used in the fields of ion detection, cell imaging, disease diagnosis, etc. This review will focus on the priciples, applications, advantages and disadvantages of molecular interaction-based fluorescence technologies and will talk about Photo-induced Electron Transfer, Intramolecular Charge Transfer, Fluorescence Resonance Energy Transfer orderly.

2. Photo-induced Electron Transfer（PET）

2.1. Principle

Photo-induced electron transfer (PET) is one of the most traditional mechanism to design fluorescence probe. Normally, a typical PET probe includes fluorophore, recognition receptor and short spacer, of which the fluorophore is conjugated with the recognition receptor via the short spacer. The recognition receptor usually has atoms that have lone pair electrons, such as N atom and O atom.

In this way, the recognition receptor can be easily combined with the target substances and can detect them easily. The PET system (Fig. 1) consists of two types of process, a-PET and d-PET, and both of them can be explained by the theory of frontier orbital theory. During the a-PET process, when the fluorophore is excited by light, the electron in the Highest Occupied Molecular Orbital (HOMO) will jump to the Lowest Unoccupied Molecular Orbital (LUMO). If the HOMO of recognition receptor is in between the LUMO and HOMO of the fluorophore, which means the HOMO energy level of the recognition receptor is higher than that of fluorophore, the electron in the HOMO of recognition receptor will transfer to the HOMO of fluorophore. Then the electron in the LUMO of fluorophore is not able to jump back to the HOMO because it has already been occupied, thus inducing the fluorescence quenching. When the analyte exists, it can coordinate with the recognition receptor and make the energy of the HOMO of the recognition receptor lower than that of fluorophore. In this case, the electron donor ability becomes weaker and the HOMO of fluorophore will not be occupied by the electron from recognition receptor, the electron in the LUMO of fluorophore can jump back successfully and the probe regain its fluorescence. Likewise, if the energy of the LUMO of the recognition receptor is lower than that of fluorophore, the electron in the LUMO of the fluorophore will jump back to the LUMO of the recognition receptor rather than the HOMO of fluorophore. The analyte can decrease the energy of LUMO of the recognition receptor to make sure the LUMO of the recognition receptor is lower than the HOMO of fluorophore. So this approach can restrict the PET process and enhance the fluorescence intensity remarkably. Since this kind of probe is like an "on-off" switch, it is also called "off–on" fluorescence sensor.

The other kind of PET probe is "on-off" fluorescence sensor (Fig. 2). In a "on-off" PET process, the fluorophore itself exhibits strong fluorescence, which means the electrons do not transfer from the recognition receptor to the fluorophore. When the recognition receptor is bound with the analytes, especially metal ions, the electrons will transfer from the fluorophore to the metal ions, then PET will be promoted and the fluorescence quenching effect will be enhanced. In general, whether the PET is "off-on" or "on-off" depends on the fluorophore, recognition receptor and analytes. Sometimes the patterns can be switched between each other, and the choice is at the request of experiment.

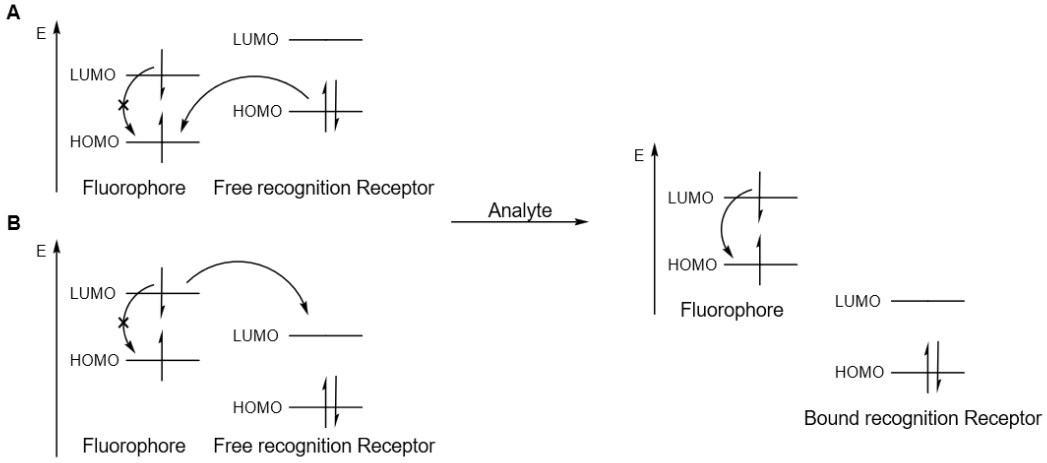


Fig. 1 Fluorescent probe based on off-on PET mechanism: (A) a-PET and (B) d-PET

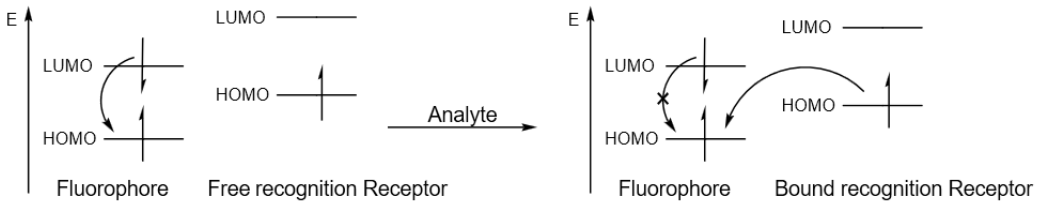
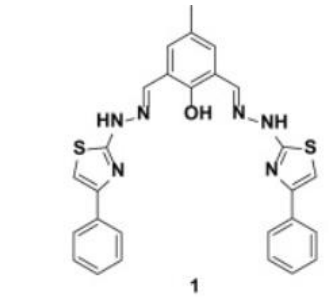


Fig. 2 Fluorescent probe based on on-off PET mechanism

2.2. Application

The most universal application of PET fluorescent probe is to detect metal ion. Metal ion plays an important role in human body life activity, and it is essential to detect and analyze their content in human body. During the past few decades, researchers have reported various kinds of PET probes for metal ions, including Cu^{+}, Zn^{2+}, Ag^{+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, and so on. Among those metal ions, the configuration of extra-nuclear electron of Cu^{+} and Zn^{2+} is $3d^{10}4s^{0}$, so they have closed shell structure. This kind of structure does not have single electron and can be used in the design of off-on PET probe to detect the fluorescence enhancement. Khan *et al.* [1] designed a weakly fluorescent chemosensor (Fig. 3, derived from [1]) and accomplish the detection of Zn^{2+}, the detection limit was as low as 2.31×10^{-8}M, which showed great sensitivity. This probe could also suppress the interference of other ions except the Cd^{2+}, because all of them did not show any fluorescence enhancement. Chang *et al.* [2] reported a highly selective dual-channel fluorescent probe, it showed strong green fluorescence due to the restriction of PET. The creativity of this probe lied in that it could detect the Zn^{2+} and PPi ions at the same time. The detection limit was 4.6×10^{-8}M, which showed great sensitivity and selectivity.

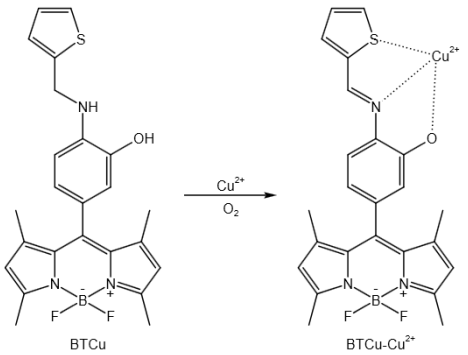


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4-methyl-2,6-bis((*E*)-(2-(4-phenylthiazol-2-yl)hydrazono)methyl)phenol

Fig. 3 The structure of the chemosensor

As to those metal ions with single electron structure like Ag^{+}, Cu^{2+} and Fe^{3+}, they all have paramagnetism, when they coordinate with the recognition receptor, the fluorescence quenching will happen. So it is not easy to enhance the fluorescence intensity and design an "off-on" PET probe. Sun *et al.* [3] designed a fluorescence-enhancement Cu^{2+} probe (BTCu) with an N, O, and S tridentate PET ligand (Fig. 4). The BTCu-Cu^{2+} probe showed excellent cell permeability and nontoxicity, but its fluorescence enhancement was very weak according to Fig. 5 (derived from [3]).



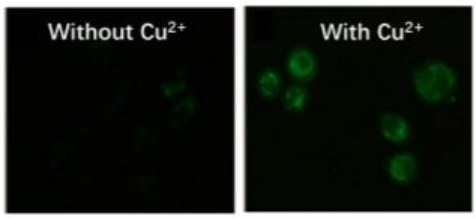


Fig.4 Chemical structure and sensing mechanism Fig. 5 Fluorescence images of HeLa cells

Hence, most of the fluorescent probes to detect this kind of metal ions are "on-off" PET probes. Wang *et al.* [4] developed a new fluorescent probe based on imidazole [2,1-b] benzothiazole to detect Cu^{2+}, in the PH range of 2-11, the Cu^{2+} resulted in the fluorescence quenching. The system was stabilized by decreasing the energy gap between HOMO and LUMO. In that case, it also strengthened the PET effect and quenched the fluorescence. The detection limit of this approach was 4.6×10^{-7} M, which showed enormous potential in the detection of Cu^{2+}.

Moreover, Li *et al.* [5] synthesized a new "on-off-on" PET fluorescent probe (Fig. 6, derived from [6]) based on Coumarin and quinolinylbenzothiazole to detect Cu^{2+}. At first, with the increase of the Cu^{2+} concentration, the fluorescence of the system became weaker. Then the PPi was added, the fluorescence intensity showed an upward trend obviously. This kind of "on-off-on" probe could repeat the detecting process for 6 times and almost showed no loss of fluorescence and could be put into detection of living cells.

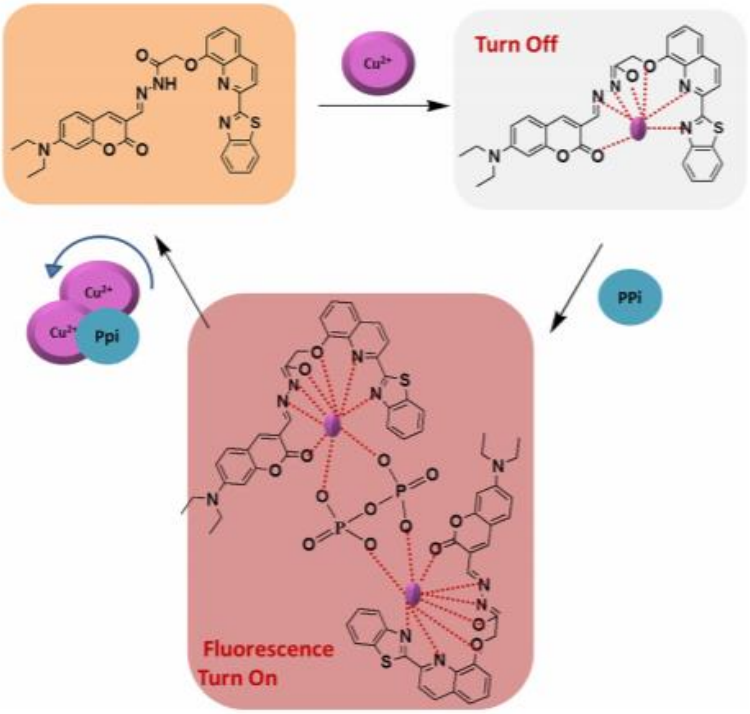


Fig. 6 The coumarin and quinolinylbenzothiazole-based ''on-off-on" type fluorescent probe

2.3. Advantages and Disadvantages

In general, the PET fluorescent probe features masses of advantages, such as high sensitivity, low fluorescence background and high signal to noise ratio (SNR) [7]. Generally, PET is favored if the oxidation potential of the ligand is less than that of the fluorophore]. However, the common disadvantage of the PET fluorescent probe is the proton interference. In most cases, proton will bind to the coordination site, hindering the PET process and enhance the intensity of fluorescence. So controlling the range of pH is very important during the PET process. Apart from that, developing new probes with lower pK_a values contributes to eliminating the proton interference. In the future, that would be the main developing direction of PET-based fluorescent probes.

3. Intramolecular Charge Transfer (ICT)

3.1. Principle

Intramolecular charge transfer (ICT) is another typical mechanism of fluorescent probe, which is based on the "Donor-π-Acceptor system". Unlike PET, an ICT probe does not have the short spacer. The fluorophore and the recognition bond with each other through covalent interaction, and then it forms a "Donor-π-Acceptor system". Such structure always leads to strong intramolecular migration. When the analyte combines with the probe, the electron donating ability of the fluorophore or the electron withdrawing ability of the receptor will be different, and the electron distribution will change, thus resulting in the spectral shift. To be exact, if the analyte is combined with the electron-withdrawing group of the probe, the electron-withdrawing ability of the electron-withdrawing group is enhanced due to the coordination of metal ions, thereby enhancing the intramolecular ICT effect,

and energy level difference between the ground state and excited state decreases and the wavelength red shift occurs. By contrast, if the analyte binds to the electron-donating group of the probe, the coordination effect of the metal ion will reduce the electron-donating ability of the electron-donating group, and the ICT effect in the molecule is weakened. The energy level difference increases, and the emission wavelength is blue-shifted (Fig. 7, derived from [8]).

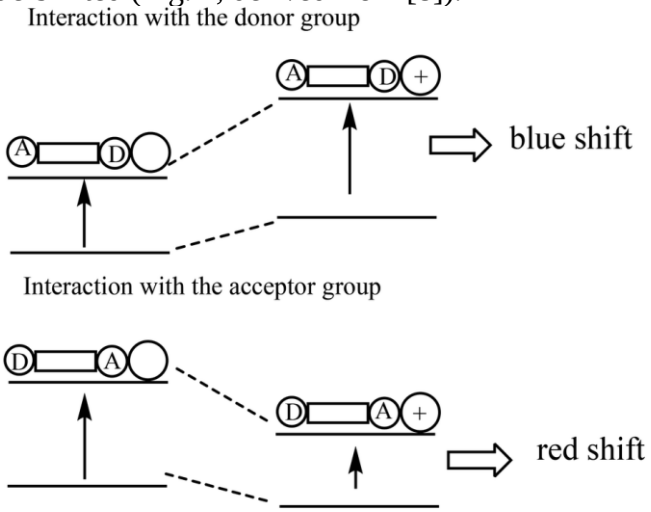


Fig. 7 Mechanism of ICT-based fluorescent probe

There is a special case in the ICT system called twisted intramolecular charge transfer [9]. In this condition, the electron donor is twisted to a state orthogonal to the aromatic ring in the original coplanar state with the aromatic ring and the system will not emit fluorescence. When restraining the process of TICT, the probe will emit bright fluorescence. Based on this principle, more and more probes are designed by enhancing TICT, including temperature sensor [10], viscosity sensor [11] and Metal ion probe [12].

3.2. Application

Since the recognition receptor always interacts with the fluorophore due to conjugation, and the fluorescence spectral shift happens after the reaction, the fluorescence quantum yield and lifetime also change correspondingly. Therefore, ICT-based probes are always applied to the design of colorimetric organic probe. Rao *et al.* [13] developed thiazole functionalized receptors to sense CN^- ions in water (Fig. 8, derived from [13]). In the reaction process, the analyte, cyanide anion, disrupted the π-conjugation through nucleophilic attack. Then it blocked the ICT process from aminophenol to benzothiozolium group, and the color of the system changed from wine red/purple to colorless. The detection limits were calculated to be 3.6×10^{-6} M and 4.2×10^{-6} M. In addition, colorimetric in-field fluoride ion sensors were reported by Samanta *et al.* [14]. They converted a norbornene-coupled 4-aminophenol derivative to a 2,4-dinitrophenyl hydrazine derivative in the norbornene-based aldehyde system (NDNP) (Fig. 9, derived from [14]). Not only did the monomer show obvious color change and large redshift in absorbance maxima, but also the homopolymer (poly-NDNP) could be used in rapid detection. This kind of ICT-based probe could detect the fluoride ion in practical toothpaste samples efficiently. Further, Munusamy *et al.* [15] designed a fluorescent probe called BBCN to detect the cyanide ions. The probe could be applied to perform ratiometric detection. For one thing, it could alter its color from green to blue after the addition of cyanide ions. For another thing, through titration experiment, the quantitative detection of CN^- with high sensitivity and selectivity was also accomplished.

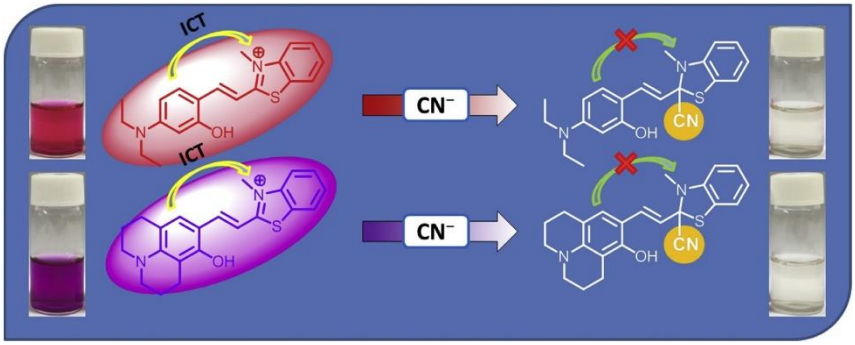


Fig. 8 CN^- detecting process based on ICT

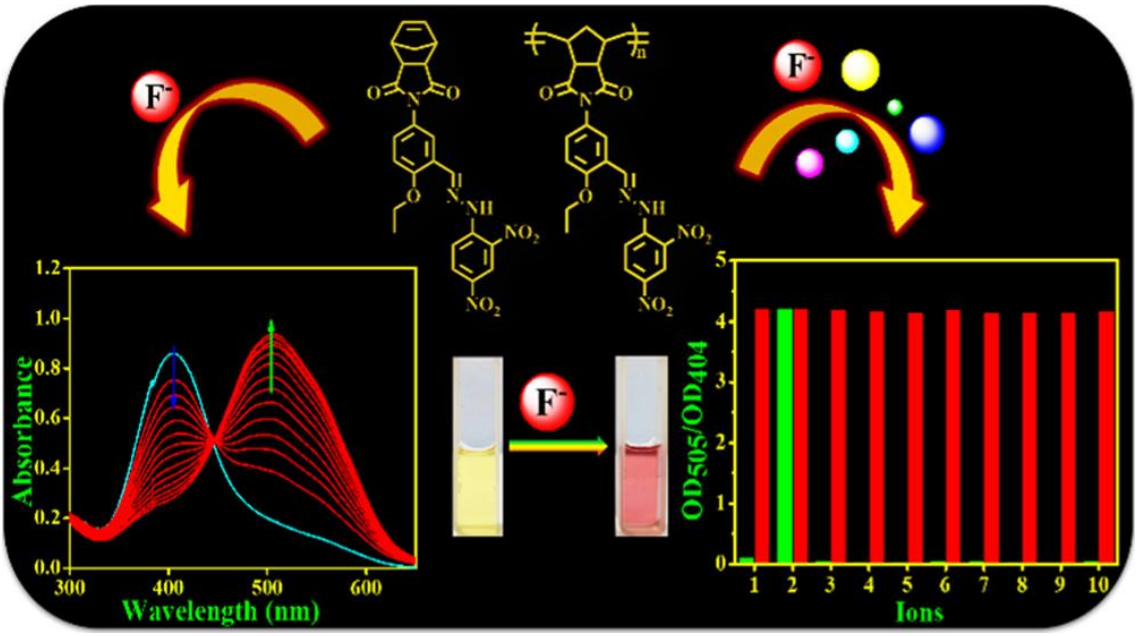


Fig. 9 Structure of the NDNP probes and the detection result

3.3. Advantages and Disadvantages

The advantage of ICT-based probes lies in its ratiometric detection. Unlike other probes based on the change of intensity, this technology can eliminate the distraction from environment and instrument, so the detecting result will be more accurate and reliable. However, there are also some problems to be solved in the design of proportional metrology probes using the ICT mechanism. Firstly, in order to achieve ratiometric detection, the shift of the emission peak should be large enough, which requires a large change in the ICT effect before and after the response. But in most cases, the emission peak of ICT is relatively wide, so it is very easy to form overlapped peaks, thus affecting the accuracy of detection. Additionally, some recognition groups have atoms with lone pair electrons, which will trigger PET effect. The PET effect will disappear after coordination, then the fluorescence intensity will change with the shift of spectrum. So sometimes the new emission peak covers the former emission peak completely and the ratiometric detection cannot be finished.

Therefore, it is very important to select and design proper fluorophore and recognition groups, but the designing strategy is not mature enough now. In the future, with the constant endeavor of scientists, more and more new fluorescent probes would be explored, which will make up for the deficiency of ICT effect.

4. Fluorescence Resonance Energy Transfer (FRET)

4.1. Principle

Fluorescence Resonance Energy Transfer (FRET) is the process where energy transfer between different fluorophores. A typical FRET-based fluorescent probe is made up of two different fluorophores, one is an energy donor and the other is an energy receptor. They are interconnected by unconjugated chemical bonds. When the probe is excited by outside energy, the donor fluorophore changes from the ground state to the excited state, resulting in fluorescence emission, and then the energy is nonradiatively transferred to the fluorophore acceptor in the ground state through dipole-dipole interactions, followed by electronic transitions and fluorescence emission of the fluorophore acceptor are generated (Fig. 10).

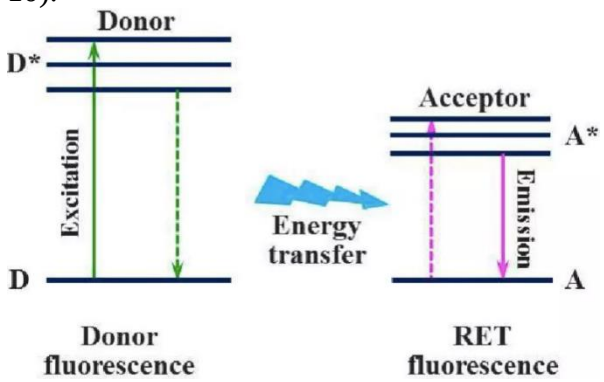


Fig. 10 Mechanism of FRET-based fluorescent probe

As to an FRET-based fluorescent probe, the donor and the acceptor must meet the following three conditons: Firstly, the emission spectrum of the donor overlaps the absorption spectrum of the acceptor to some extent, in which case the energy can transfer from donor to acceptor. Secondly, the distance between the donor and accptor should be proper. Under normal circumstances, the value of the distance is no more than 10^{-8} M. Ultimately, the resonance directions of the donor and acceptor molecules must be parallel or resonance-parallel. Usually, by adapting the first two conditions, the efficiency of FRET will show remarkable changes.

4.2. Application

Nowadays, various kinds of FRET-based fluorescent probe have been put into ion detection, including cations and anions. Compared with those based on PET and ICT, FRET-based fluorescent probes exhibit properties which are more ideal for ratiometric sensing. As to the cations, Li *et al.* [16] reported a FRET-based two-photon fluorescent probe which could detect Pd^{2+} in living cells with ratiometric method. They chose 7-substituted coumarin group and Rhodamine B as the energy donor and energy receptor respectively, and then synthesized a ratiometric two-photon fluorescent probe (RN3) (Fig.11, derived from [16]). The UV–vis and fluorescent spectra shew that without Pd^{2+}, the maximum absorption wavelength was 365 nM, but when the Pd^{2+} was added, the intensity absorption increased tremendously in the range of 530–600 nm. This probe was applied in the detection of zebrafish *in vivo*, and the result displayed high sensitive and selective signal with low cytotoxicity. Chung *et al.* [17] introduced a first-generation ratiometric FRET copper probe called FCP-1, which could be an effective reliable approach to detecting the labile Cu^{+}. By regulating FRET between fluorescein donor and rhodamine acceptor units in a dose-dependent manner, the specificity of Cu^{+} detection would be increased. For the anions detection, a new two-photon excited FRET probe which could detect HSO_3^-/SO_3^{2-} was reported by Yang *et al.* [18]. The 2-acetyl-6-dialkylaminonaphthalene (acedan) moiety was selected as energy donor while hemicyanine was the energy receptor (Fig.12, derived from [18]). This probe could be exited at long wavelength, which quite reduced the background noise and cell damage.



Fig. 11 The structure of the FRET-based two-photon fluorescent probe and fluorescence image

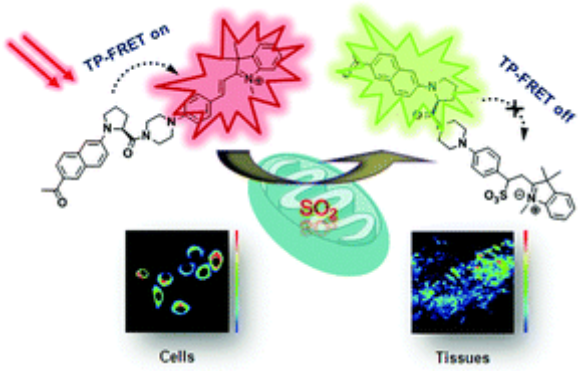


Fig. 12 The two-photon excited FRET probe and fluorescence image

Additionally, FRET-based fluorescent probes can also go for biomacromolecules like protein, DNA, RNA, and so on. Those biomacromolecules all have large size and conformational flexibility, some even have secondary structures and tertiary structures. Disease development and progression are often associated with abnormal protein folding. During the protein detecting process, fluorescence lifetime imaging microscopy (FLIM) is always combined with FRET effect, because measurement of the donor fluorophore lifetime is helpful to the calculation of FRET efficiency. Up to now, the FLIM-FRET fluorescent probes have been used in detection of cancer-metabolism-related proteins [19] [20].

4.3. Advantages and Disadvantages

The advantage of FRET technology lies in its ratiometric sensing, because the shift between two emission peaks is larger and the overlap is small, so it is easy to do ratiometric detecting. However, this large shift also causes large background noise. The FRET-based fluorescent probes are always sensitive to the changes of external environment, such as PH, ion concentration and temperature. So the biggest limitation of this technology is the low SNR value associated with fluorescence imaging. Now many technologies like filter set-based, acceptor photobleaching and FLIM have been explored, but their SNR is still far from satisfactory. In the future, research on FRET-based fluorescent probes should focus on improving quantum efficiency and FRET-transfer efficiency. Apart from that, designing more sensitive and selective probes and increase SNR are also indispensable.

5. Conclusion

In summary, three kinds of molecular interaction-based fluorescence technologies, including their mechanisms, applications, advantages and disadvantages have been reviewed in the article. It is delightful to see that with the development of technology, more and more fluorescent probes for different purposes have been developed by researchers. Besides the three technologies mentioned in the article, there are some other molecular interaction-based fluorescence technologies such as Excimer-Exciplex (ME), Excited-state Inter‑molecular Proton Transfer (ESIPT), Aggregation-induced Emission Enhancement (AIEE), all become widely applied. An ideal fluorescent probe is

supposed to meet the following conditions: chemical stability, light stability, PH stability, selectivity and sensitivity, quick response speed, hypotoxicity and long excitation wavelength. Nevertheless, it is still difficult to invent a fluorescent probe that has the above conditions at the same time. Now the challenges are as follows: To begin with, some fluorescent probes for active small molecules are not quick enough to respond to the target molecules, because the half life period of active small molecules are short in body. Next, most current fluorescent probe imaging stays at the cellular level, research about subcellular organelle is still limited. Finally, how to increase SNR and decrease detection limit is still a difficult problem for current research.

In general, with the deepening of the research on the physiological functions and pathological effects of various biological and inorganic species in the living system, there is an urgent need to develop brilliant probes targeting these substances with excellent performance. Meanwhile, it is important to base on the characteristics of different analytes and design new strategies to solve the difficulties and challenges encountered in current research.

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