ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

A SHORT REVIEW ON THE RECENT ADVANCEMENT OF FLUORESCENT PROBES FOR FORMALDEHYDE SENSING 2017 ONWARD

Krishanu Sarkar

Netaji Mahavidyalaya, Arambagh, Hooghly, West Bengal, India

**Corresponding author: krishanu79@gmail.com*

ABSTRACT

Formaldehyde is not only a ubiquitous chemical pollutant in indoor environments but also a reactive carbonyl species in biological processes. Formaldehyde is endogenously produced through the essential biological processes, including mitochondrial one-carbon metabolism, metabolite oxidation, and nuclear epigenetic modifications. High electrophilic property allows it high reactivity with a wide variety of biological nucleophiles, which can be beneficial or detrimental to cellular function depending on the situation. Therefore, it is important to develop non-invasive sensing technique for monitoring the roles and functions of FA in biological processes. So, development of a rapid, sensitive and facile method to determine the concentration of FA becomes highly desirable. Fluorescence detection technique is highly attractive because of its high selectivity, sensitivity and real-time approach. This review article focuses on various design strategies of the fluorescent probes, their sensing mechanism for detecting formaldehyde in environment as well as in living cells based on different recognition groups from 2017 onward. This area still in prime focus because of the rapid development of the fluorescence probes from lab to indoor in near future.

Keywords: Fluorescent Probe, Sensitivity, Selectivity

1. INTRODUCTION

The simplest aldehyde, formaldehyde (FA), an endogenously produced reactive carbonyl species (RCS), has attracted significant attention in the field of science because of its potential applications in various fields such as cosmetics [1], plastics [2], drugs [3] and industrial chemicals [4]. In addition, FA has been recognised as the third largest indoor chemical pollutant and toxin which is released from plywood manufacturing and vehicle exhaust [5]. Because of its amphibolic nature FA easily enters into the cells as a carcinogen and causes DNA damage by reacting with nucleophilic material actively [6]. Exposure to FA may cause diseases by the combination with DNA. Short-term exposure of FA can cause headache [7], tingling sensation in the throat [8], dyspnoea and its long-term exposure may cause memory loss [9], cancer [10] even death [11]. Moreover the Alzheimer's disease is related to the intake of FA [12]. In 2004 International Agency for Research on Cancer (IARC) has categorized FA in Group I, carcinogenic [13] to humans whereas China has ranked it second in the control list of chemicals toxic to humans [14]. The United States Environmental Protection Agency suggested the limit of FA as 0.2 mg kg^{-1} of body weight in daily life and WHO set it as 0.15 mg kg^{-1}. In spite of being hazardous product, FA exists in all cells and plays a vital role in the carbon cycle of metabolism. In living systems, endogenous FA may be generated in many biological processes such as one carbon metabolism [15], various demethylation events [16] or methylation metabolism of methylation of DNA [17]. In a normal physiological brain, the concentration of formaldehyde ranges from 0.2 mmol to 0.4 mmol. At this level, formaldehyde is essential to the memory formation via DNA demethylation cycles and cognitive ability [18]. However, the physiological function of FA is still not clear. Moreover, FA certainly performs as a key signalling molecule in the course of disease development and may be a target for drug release. Therefore, FA acts as a double role not only in public health but also in industrial development. Thus, it is very important to develop facile, selective and reliable methods to detect FA for environmental monitoring and biological study.

At present several methods have been used for the detection of formaldehyde including piezoelectric sensors [19], electro-chemical biosensors [20], quartz crystal microbalance [21] Raman spectroscopy [22] colorimetric assay [23] gas chromatography [24] mass spectrometry

[25] and high-performance liquid chromatography [26] etc. Each detection method has its own limitations and obviously sensor technologies [27] are indispensable for in situ and real-time detection of FA. Compared to semiconductor based sensors [28] fluorescent sensors have advantages because of its simplicity, non-destructiveness, biocompatibility, high sensitivity [29] and quick response [30].

Selective in situ detection and quantization of FA in living cell and tissue has become a new challenge and that requires a real-time and non-invasive technique. As molecular imaging practice relies on target-specific probes for *in vitro* [31] and *in vivo* [32] detection and quantization of biomolecules, it is widely applied for accurate detection, location and propagation of the disease [33]. Definitely the molecular imaging technique can be applied clinically in the discovery and development of novel drug therapy, image guided surgery [34] through real-time assessment [35]. In recent years, the development of fluorescent probes for metal ions, anions and biomolecules has made significant progress in sensing FA. Specially small molecule fluorescent probes have widen up different strategies starting from single emission [36] to ratiometric fluorescence [37], visible-light emission [38] to near-infrared emission [39] single photon excitation [40] to two-photon excitation [41].Therefore, fluorescent probes based on small organic molecules for the detection of FA have gained prime interest in recent years [42]. Particularly over the past three years, several formaldehyde-responsive fluorescent probes have been developed and used in monitoring the FA level in environment and biological samples. Keeping in mind the rapid development in the field of FA responsive fluorescent probes, here the recent progress and future strategies in this field has been summarised.

2. CLASSIFICATION OF FLUORESCENT FORMALDEHYDE PROBES

Design of effective fluorescent probes for imaging FA in living cells and specimens must include prompt reactivity with FA and its selectivity against other biological analytes which possess similar electrophilic carbonyl groups. According to the sensing products, FA fluorescent sensors can be classified into two categories: (1) formimine or formimine intermediate from the primary amine probes including (i) aza-Cope rearrangement and subsequent β-elimination (ii) aromatic amine (iii) aromatic hydrazine and (2) aminal from o-diamino probes shown in Fig. 1. In these paths FA detection can be achieved via various sensing mechanisms, such as photo induced electron transfer (PET), spirocyclization, intramolecular charge transfer (ICT), as well as various response modes, such as fluorophore uncaging (turn-on) and ratiometric detection.

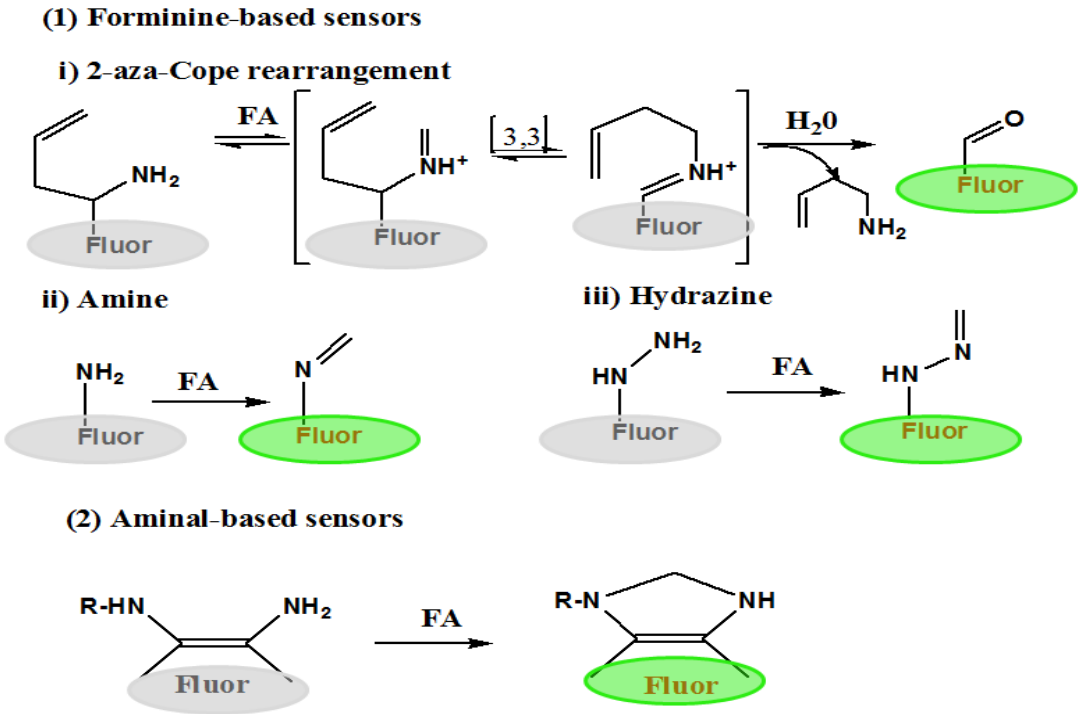

Fig. 1: Different reactive sites of fluorescent probes and sensing mechanism for FA

2.1. Forminine based fluorescent probes

Forminine or forminine intermediate based fluorescent probe for FA sensing includes 2-aza-Cope rearrangement and subsequent β-elimination, aromatic amine and aromatic hydrazine based chemical reaction. Many reports of forminine based fluorescent probes were found in the literature but here those reports which comes mainly in last three years will be discussed.

Chang and his group [43] have reported a FA sensing protocol in living cells (Fig. 2) that involves 2-aza-Cope reaction of a homoallylamine trigger by a self-immolative β-elimination linker that was grafted into a fluorophore moiety having a phenol group (Probe 1) and opens a new horizon for a wide range of fluorescent scaffolds for FA detection.

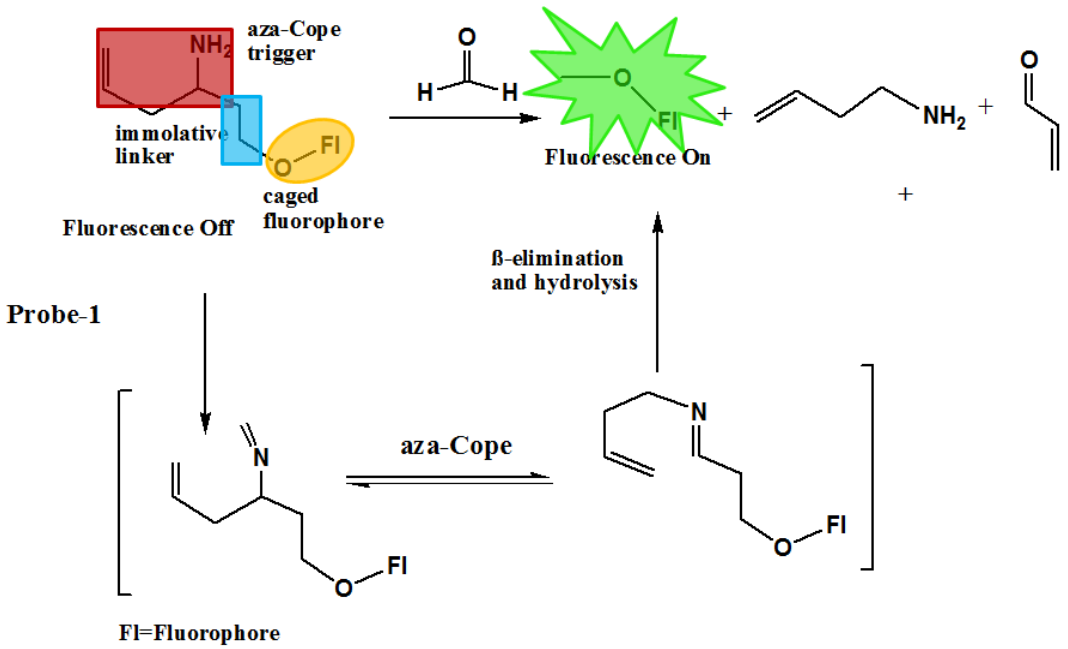

Fig. 2: Self-immolative aza-Cope strategy for FA responsive fluorescent probe-1

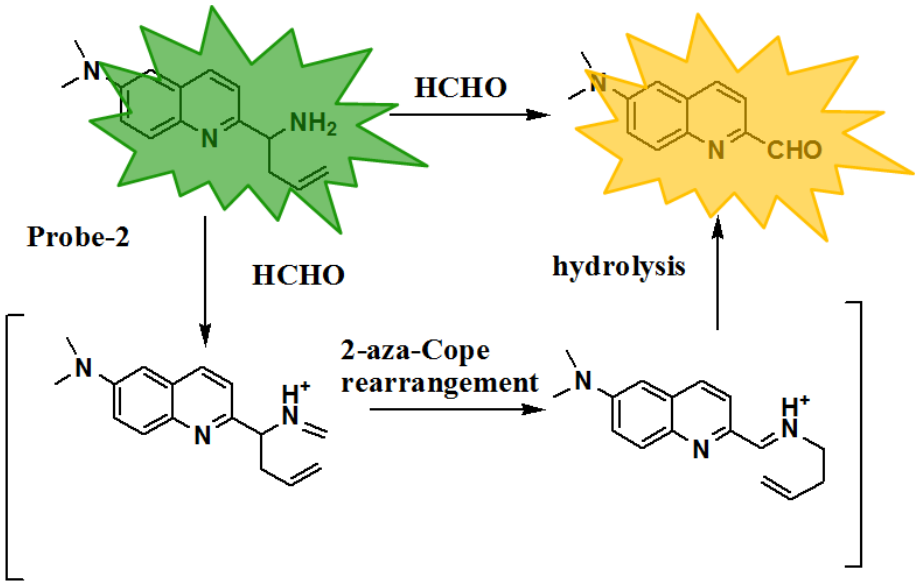

Fig. 3: The structure of Probe-2 and the proposed ratiometric FA sensing mechanism

As this trigger is potentially applicable to any alcohol or amine based fluorophore scaffold by direct attachment or via self-immolative linkage, this strategy finds potential application in elucidating novel sources and targets of FA metabolism in biological field.

Quan and his co-worker [44] has developed a novel ratiometric fluorescent probe (Probe 2) for endogenous FA sensing (Fig. 3) in live plant tissue of *Arabidopsis thaliana* for the first time. They choose N, N-dimethylquinolin-6-amine as the fluorescent chromophore because of its excellent photophysical and chemical properties. The sensing mechanism of this probe 2 follow 2-aza-Cope rearrangement reaction and showed a major red shift (75 nm) in the emission profiles and a sharp change of the fluorescence signal ratio during interaction with FA. Thus this highly selective and effective probe 2 finds potential applicability for monitoring FA in real water samples and other plant tissues and will allow better understanding of its metabolism mechanism in FA related physiological and pathological aspects.

Meanwhile You and his team [45] reported a bright sensitive ratiometric fluorescent probe 1-(4-(1H-phenanthro [9,10-d]imidazol-2-yl)phenyl) but-3-en-1-amine (Probe-3) for imaging endogenous FA in cells, zebrafish, and the renal tissue of a living mouse. This Probe-3 exhibited high quantum yield (Φ=0.62) in blue fluorescent emission and after selective reactivity toward FA emits bright green fluorescence (Φ=0.51).

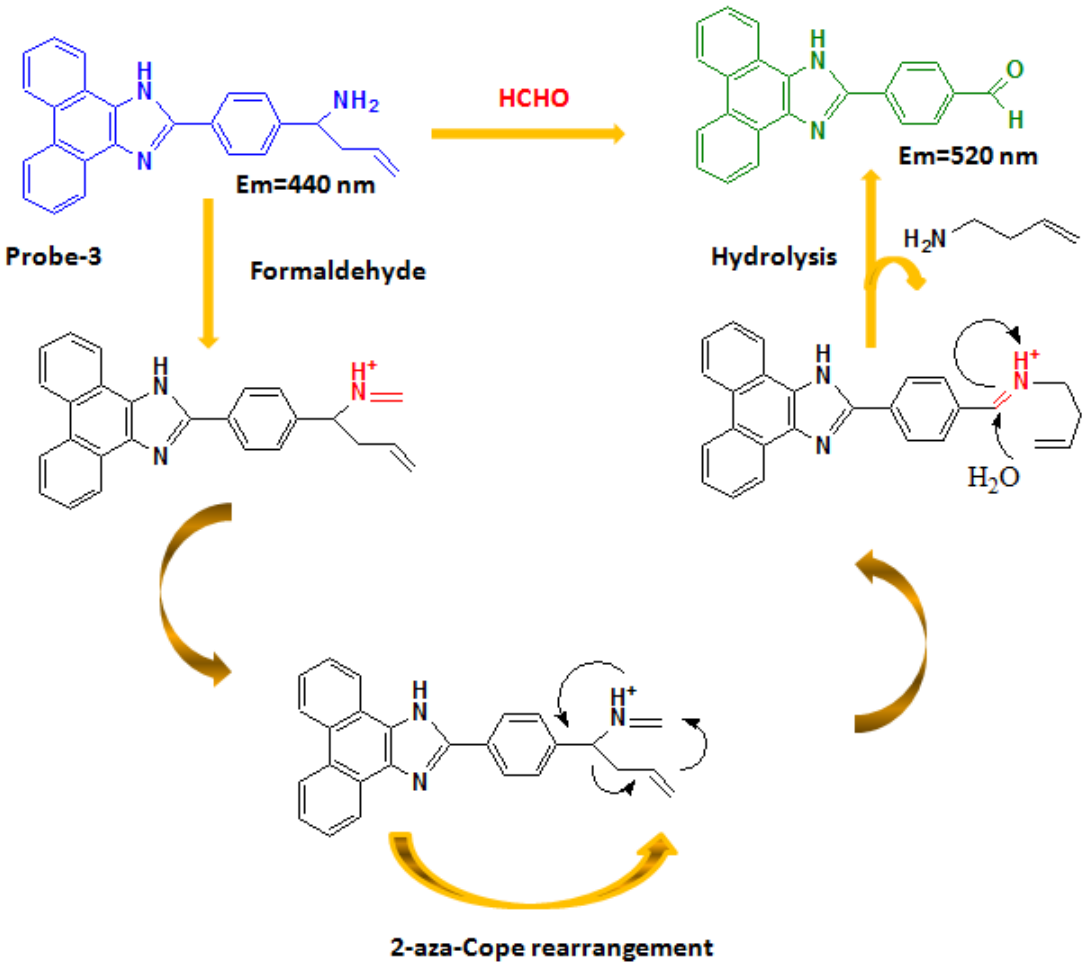

Fig. 4: The sensing mechanism of Probe-3 and the proposed sensing mechanism

The probable sensing mechanism of Probe-3 involves first the imine ions formation, then 2-aza-Cope rearrangement, and finally hydrolysis (Fig.4). When sensing FA, this probe showed large red shift (80 nm) in emission wavelength and the huge ratiometric fluorescence enhancement (92.2-fold) as well as a quite low detection limit (0.84 mM). Furthermore they extended their experiment in detecting FA in both HeLa cells and renal tissue of a living mouse and presented a breakthrough in FA imaging and exploration in living systems.

Later in 2018, Zheng *et al*. [46] developed a highly selective, sensitive and ratiometric fluorescent probe (Probe-4) for FA tracing in various medium, such as

aqueous solution, serum and air based on aza-Cope reaction. They use 2-(2- hydroxyphenyl)benzothiazole as the dyes platform and pro-aza-Cope rearrangement moiety for FA (Figure-5). From the figure it is clear that Probe-4 undergoes excited state intramolecular proton transfer (ESIPT) reaction and produce keto form of probe-4 which on interacting with FA via an aza-Cope rearrangement produce an electron deficient aldehyde group where a strong intramolecular charge transfer (ICT) process will lead to a remarkable red-shift (79 nm) of the fluorescence bands of the probe, and afford ratiometric signals (39 fold enhancement). This group successfully applied Probe-4 for the visual (from blue to yellow) detection of gaseous FA.

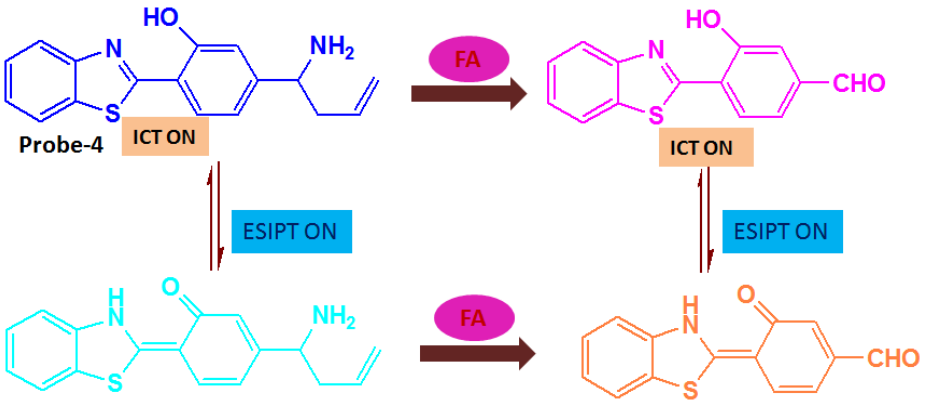

Fig. 5: Schematic diagram for FA sensing of the fluorescent probe-4

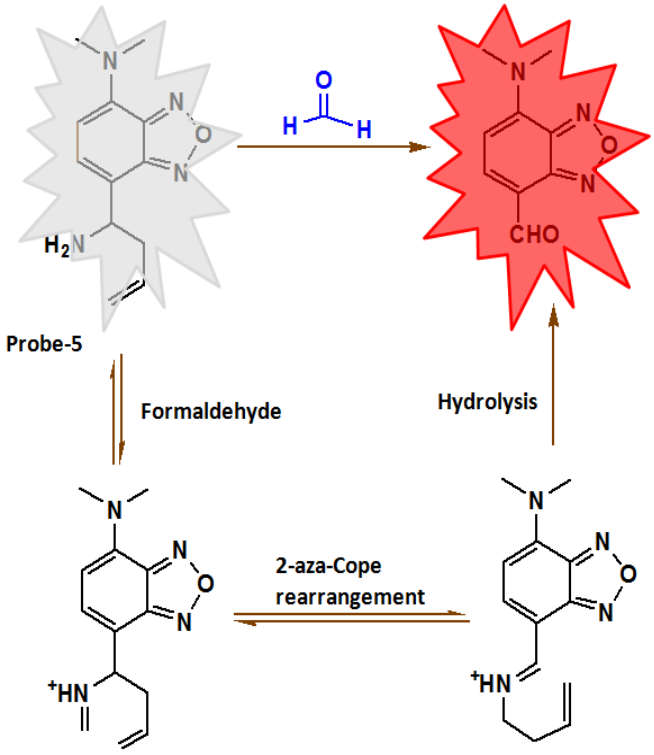

Fig. 6: Design and mechanism of the fluorescent probe-5

Based on the same sensing mechanism Wang [47] group reported an excellent fluorophore probe benz-2-oxa-1, 3- diazole (Probe-5) for endogenous or exogenous FA detection in living cells and tissues over other reactive carbonyl species. Here also first homoallylamine moiety of probe-5 reacted with FA to produce 2-aza-1,5-dienes, and then the 2-Aza-Cope rearrangement occurred via [3,3]-migration to form α, β-ene, which was finally hydrolyzed in aqueous solution to release highly fluorescent aldehyde compound (Fig. 6). During interaction with FA, the fluorescence intensity was significantly increased (55-fold) and exhibited large Stokes shifts (about 118 nm). Moreover for the first time this probe was successfully applied to monitor exogenous FA changes in *Daphnia magna* through fluorescence microscopy, indicating its potential application for biological processes.

In the same year Yin and his group [48] newly designed a turn-on fluorescent probe, tetraphenylethylene (TPE) derivative (Probe-6) based on aggregation-induced emission (AIE) mechanism for facile detection of gaseous FA. To get the best result of the probe-6, 4-nitrobenzyl (Ph-NO_2) group, a fluorescence quencher, was incorporated into TPE (Fig. 7). Initially, this probe has a weak fluorescence (fluorescence quantum yield, Φ=0.03) due to photoinduced electron transfer (PET) process between the electron donor and the electron acceptor. However, after reacting with FA, weakly emissive probe turns into a highly emissive compound (fluorescence quantum yield, Φ=0.35). Moreover, the

detection limit for FA sensing is 0.036 mg/m^3 which is lower than the air quality guideline value of gaseous FA (0.1 mg/m^3) recommended by World Health Organization (WHO). As this probe can detect gaseous FA in solid state, this probe can be used as a safer and convenient portable sensor compared to solution based sensor.

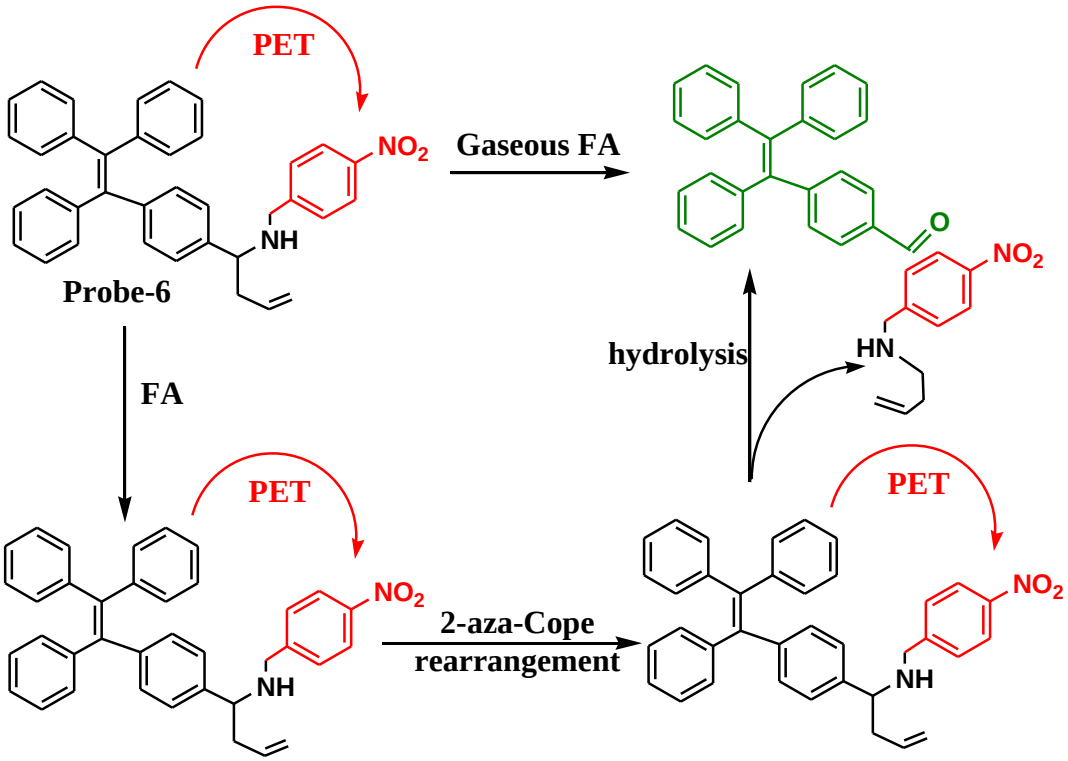

Fig. 7: Schematic diagram and FA sensing mechanism of the probe-6

Lv and his team [49] first time synthesized a reversible fluorescent probe (Probe-7) for the selective and sensitive detection of FA in living cells and in vivo more precisely. The probe employed a BODIPY fluorophore as the fluorescence signal transducer and a primary amino group as the fluorescence modulator.

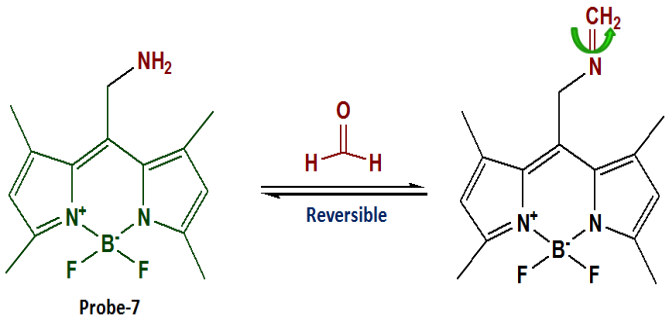

Fig. 8: The structure of the probe-7 and the reaction mechanism for FA detection

The probe can proceed intracellular aldimine condensation reaction with FA and form imine (C=N) which will result in C=N isomerization and rotation to turn-off the fluorescence of the probe (Fig. 8). The probe in the free state exhibited strong fluorescence emission (fluorescence quantum yield, Φ=0.85) and after reacting with FA the fluorescence quantum yield reduced to Φ = 0.01. The probe could selectively and sensitively detect FA in a reversible manner with a low limit of detection of 50 nM. The probe has been successfully applied to detecting and imaging FA in different living cells and thus can be used as a potential chemical tool for FA sensing in biological study.

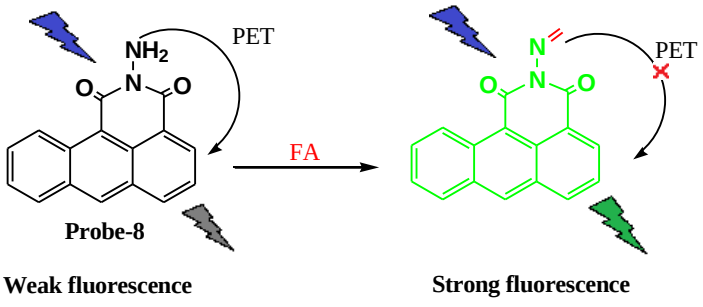

Fig. 9: The proposed sensing mechanism of the probe-8 for FA

Later anthracene carboxyimide-based fluorescent probe (Probe-8) was developed by Wang group [50] for the detection of toxic FA. The fluorescence process involves an aldimine condensation reaction with FA, and a noticeable fluorescence "turn on" signal (Fig. 9). The probe shows a weak emission fluorescence (Φ = 0.015) at λ=518 nm because of photoinduced electron transfer (PET) from hydrazine to the anthracene imide moiety. Upon addition FA, the significant fluorescence enhancement occurs (Φ = 0.041) that corresponds to inhibition of PET process. The limit of detection for FA sensing of this probe was found to be very low (988 nM). In addition, cell imaging results indicated that the probe can examine exogenous formaldehyde in HeLa cells with remarkable fluorescence turn-on signal. Therefore, this probe has potential applications for the detection of FA in biological systems.

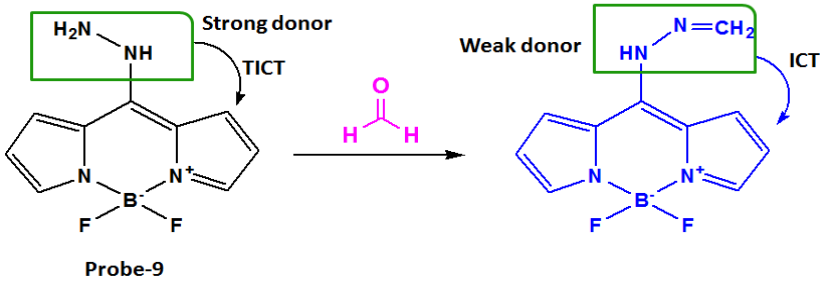

Fig. 10: Design and sensing mechanism of the probe-9

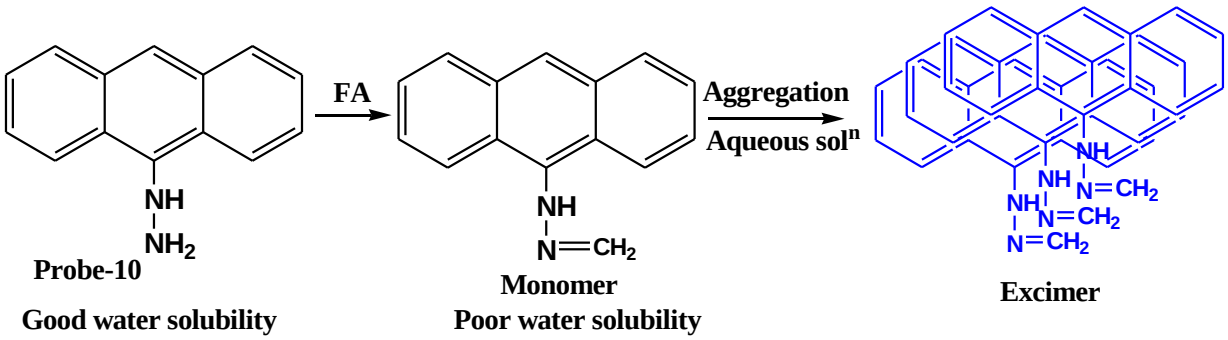

Fig. 11: Design and sensing mechanism of the probe-10

Recently a fluorescent BODIPY substituted hydrazine probe (Probe-9) was introduced by Song *et al.* [51] for FA detection. The probe was synthesized by the nucleophilic substitution reaction of a meso-chlorinated BODIPY dye with hydrazine. The probe can react with FA efficiently and form a hydrazone derivative, blue fluorescent compound, giving a very large turn-on fluorescence response (>900- fold increment). The sensing mechanism has been attributed to the switch of the intramolecular charge transfer (ICT) process from a twisted ICT to ICT (Fig. 10). The probe shows fast response to FA (<30 min) and has a low limit of detection (0.18 μM). The fluorescence quantum yield of the FA sensing product was measured as 0.40 with compound probe. Moreover, this probe shows very low cytotoxicity and can detect endogenous FA in live HeLa cells by fluorescence images.

On the other hand Hou group [52] reported a novel fluorescent probe (Probe-10) to monitor FA based on anthracene by applying one-step Buchwalde Hartwig amination reaction. They simply introduce formaldehyde recognition unit (hydrazine group) into anthracene ring (fluorophore). As shown in Fig. 11, probe-10 (9-hydrazino-anthracene), due to the presence of hydrazine group showed good water solubility and has no visible fluorescence. But after interaction with FA the monomeric product aggregate readily due to poor water solubility and shows strong blue fluorescence because of the monomer-excimer interaction. The probe upon interaction with formaldehyde, fluorescent emission around 445 nm increases about 200-fold in 5 min in comparison to free probe. Due to low limit of detection (0.23 mM), excellent pH stability, high selectivity, good biocompatibility and low cytotoxicity this probe can be used for imaging formaldehyde in living cells.

For the first time Lin *et al.* [53] reported the synthesis and application of an FA-selective fluorescent probe (Probe-11). This was the first endoplasmic reticulum (ER) targeted fluorescent probe for FA. The presence of methyl sulfonamide moieties in the probe makes it cell permeable and can preferentially accumulate in the ER. They choose 1, 8-naphthalimide as the fluorescent chromophore and a hydrazine as the interaction site as a synthetic strategy. The probe display almost no fluorescence because of photo-induced electron transfer (PET) process from hydrazine to 1, 8-naphthalimide.

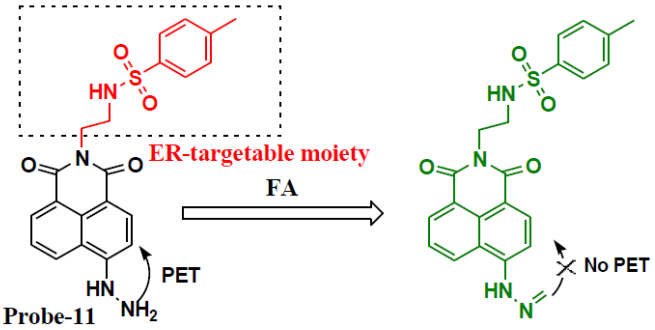

Fig. 12: Design and sensing mechanism of the probe-11

But after reaction with FA, a condensation reaction between hydrazine and FA occurs and simultaneously PET process is inhibited exhibiting a significant turn-on signal (Fig. 12). Additionally, the probe enabled the bio-imaging of exogenous and endogenous FA in living HeLa cells. Most significantly, the novel probe was capable to visualize the endogenous FA in the ER in living cells for the first time.

2.2. Aminal based fluorescent probe

Another reactivity-based approach for FA detection exploits aminal-forming properties between FA and amines. This approach involves fast reaction kinetics but is more challenging to tune for FA selectivity over other reactive species and thus offering distinct advantages and disadvantages over the forminine based strategy.

A novel aminal-based fluorescent probe (Probe-12), an ortho-diaminorhodamine derivative, for discrimination and detection of FA from other aldehydes has been developed by Zeng and his team [54]. This reaction-based probe showed distinct turn-on emission patterns upon interacting with FA. Detection principles are based on the reaction kinetics between the probe and FA as well as the difference of the fluorescence properties of the product (Fig. 13).

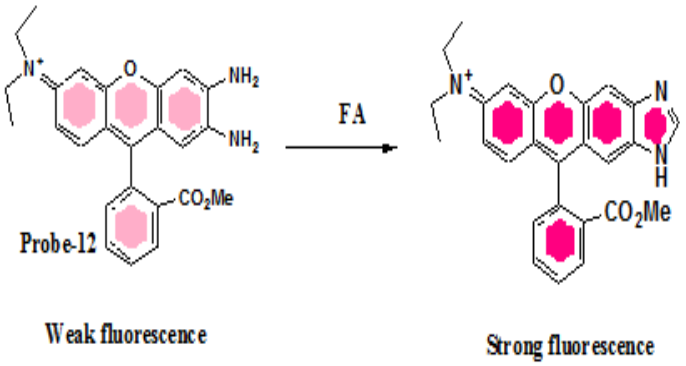

Fig. 13: Design and sensing mechanism of the probe-12

Again due to its unique turn-on fluorescence response by single wavelength excitation, the probe can be used for imaging of FA in living cells.

Based on boron dipyrromethene and o-phenylenediamine (OPDA) a new highly selective fluorescent chemosensor Bodipy-OPDA (Probe-13) for FA has been synthesized (Fig. 14) by Guo and his co-worker [55].

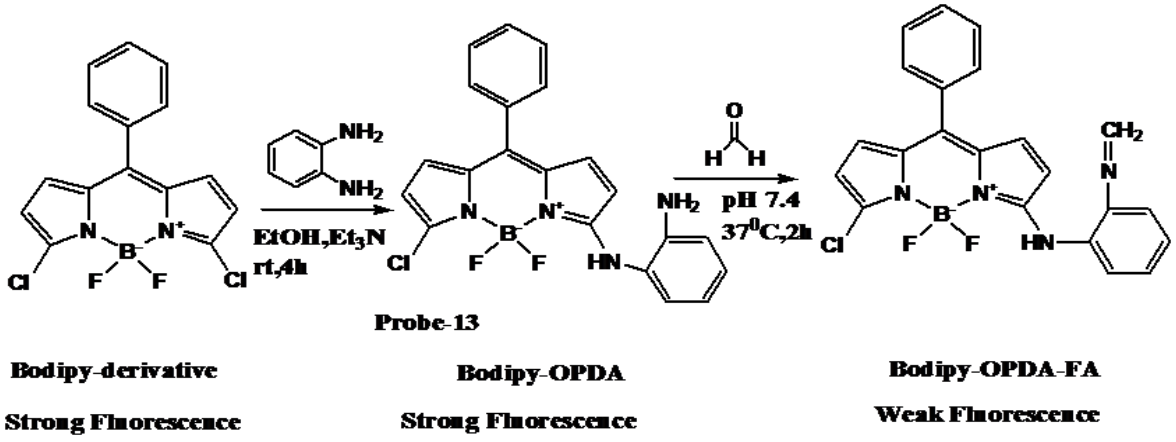

Fig. 14: Design and sensing mechanism of the probe-13

During FA sensing, the fluorescence emission band of the chemosensor observed red shift from 525 nm to 548 nm and an increase fluorescent intensity with strong green fluorescence. This probe also exhibited the low detection limit (0.104 μM). Both the cellular fluorescence imaging study and test papers detection confirmed that the probe was highly responsive to the FA in endogenous cells as well as in the gaseous environment.

3. CONCLUSIONS

In this review, the recent advances in formaldehyde-responsive fluorescent probes have been summarized. Here the reported formaldehyde responsive fluorescent probes mainly introduced two strategies (1) formimine or formimine intermediate from the primary amine probes including (i) aza-Cope rearrangement and subsequent β-elimination (ii) aromatic amine (iii) aromatic hydrazine and (2) aminal from o-diamino probes. In most of the cases FA detection can be achieved via various sensing mechanisms, such as photoinduced electron transfer (PET), spirocyclization, intramolecular charge transfer (ICT), as well as various response modes, such as fluorophore uncaging (turn-on) and ratiometric detection. In last two years some novel fluorescent probes which enable real-time, in situ detection of FA in living cells are reported. But to the best of our knowledge, there is no report about an approach that can detect them in clinical trials. Therefore more studies focusing on FA sensing in clinical trials need to be conducted in future. Moreover, the emission wavelength of most of the fluorescent probes locating in the visible region limited their further application in vivo. So, future efforts will focus on developing near-infrared fluorescent probes that helps deeper tissue penetration and reduce interference from background fluorescence.

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