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Use of Fluorescent Probe for the Fast and Quantitative Detection of Chemicals in the Blood

Abstract

Different methods are adopted for the quantitative detection of chemicals in the blood. One of which is discussed here, i.e., the fluorescent probes that are preferred in biological systems owing to their sensitivity, high selectivity, elevated spatiotemporal resolution, and non-invasiveness. In recent years, for the identification of various chemicals, a large number of probes have been designed. For the detection as well as identification of fluorescent probes, sulfides, different chemicals such as iron, glucose, glutathione etc., in the blood are discussed. The maximum detection limits of newly synthesized fluorescent probes for different chemical analysis are studied and comparatively analyzed. Different parameters like pH, incubation time, mixing time, the detection limit of the fluorescent probes is also explored.

Key Words: Fluorescence, Fluorescent Probe, Fluorophore, Detection Limit, Hydrogen Sulphide, Iron, Glucose, Glutathione

Aims and Objectives

The main aim of this review is to have knowledge about the proper working of fluorescent probes and their diagnostic and therapeutic role in the determination of chemicals in the blood. An overview is provided about the recent development of fluorescent probes in science research. It is an important aspect to share information and receive feedback from clinical experts, health professional and bioengineering experts to develop modern techniques and strategies. Combine collaboration with these experts is very important.

Introduction

With the advancement of time, many complex diseases were discovered, so new and modern detection techniques were required. One of the most effective and widely used techniques used for the detection of biomolecules is the Florescence dye probe (Masilamani et al., 2004).

Fluorescent probes are the molecules bound by chemical interactions assist in the detection of biomolecule as protein, amino acid, and antibody. They absorb light at specific wavelengths and emit at different. They provide qualitative as well as quantitative data regarding biochemical and physiological properties in the biological specimens. The most common Fluorescent probes are Fluorescein, Green fluorescent protein, and Ethidium bromide. The fluorophore is the resultant reactive derivative of the fluorescent molecule. The 2 important properties of the probe are (a) ability to determine different sites in the protein, cell membrane etc., (b) fluoresce efficiency, which is determined by the physical properties of the cell membrane etc.

The fluorescence probe technique is a new physicochemical approach for the quantitative and efficient identification or detection of chemicals in the blood (Leung et al., 2013).

Types of Fluorophores

Fluorophores are often classified into three types: (a) Biological fluorophores (FL), (b) Organic dyes and (c) Quantum dots (QDs) Every fluorophore consists of dynamic properties, and upon these properties, the

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fate of fluorophore is decided which one is suitable for different applications.

Organic Dyes

Fluorescein is the organic dyes (synthetic) that were the primary fluorescent compounds used in the research project, and to reinforce the solubility and photostability of these organic dyes; their derivatives are developed. Derivation of these organic dyes is done to further applications in bioconjugation. In bioconjugation strategies, these fluors have more advantages over the biological fluorophores because of their unique features such as small size, easy to crosslink to the macromolecules like antibodies, avidin and biotin, and showing no interference with the other biological functions.

Biological Fluorophores

In the 1990s, biological FL was used in research projects for the very first time when cloning of green fluorescent protein (GFP) was obtained as a result of cloning of jellyfish Aequorea Victoria. Afterwards, GFP derivatives, phycobiliproteins (phycoerythrin, phycoerythrocyanin, allophycocyanin and phycocyanin) as well as other proteins, are developed to be used in biological systems, and their vital use in different research projects are now common. These types of fluorophores offer an advantage to introduce expression plasmids into whole individuals, cells, organs, or bacteria, to improve the overall expression of fluorophore in the biological processes understudied (Kumar & Pal, 2016).

Quantum Dots

Quantum dots (QDs) are nanoscale materials that can transport electrons. Emission of light of various wavelengths and colors occurs when Ultraviolet radiations come in contact with these semiconducting nanoparticles. Because of their attractive and efficient properties, these nanoparticles have gained importance in fluorescent biological labels, solar cells, and composites. In 1981, Alexey Ekimov discovered these semiconducting nanoparticles in glass matrixes, and from the 1990s, these particles have given extensive attention for the utilization in fluorescence during scientific research (Chandan et al., 2018).

For theranostic applications, these nanostructure-quantum dots QDs have paved their ways to be active as a nanocarrier in fluorescent labels (Matea et al., 2017).

Most Used Fluorescent Probes

Green Fluorescent Protein (GFP)

GFP is the protein that is made up of amino acids that produce bright green fluorescence when exposed to the blue region of ultraviolet radiations. Aequorea Victoria is its main source that is a marine jellyfish. In biotechnology, GFP has a significance to develop internal chromophore without the requirement of any auxiliary gene products, cofactors etc. It was discovered by Osamu Shimomura and Roger y. Tsien. In order to check the toxicity levels of various chemicals such as paraben (derivatives of benzoic acid), triclosan, ethanol and formaldehyde, GFP acts as a reporter protein. It is also used to measure the onset and termination of calcium signalling in the cytoplasm, nucleus, and endoplasmic reticulum (Kumar & Pal, 2016).

Ethidium Bromide

It is an orange-red Fluorescent probe used to visualize nucleic acid as it binds to DNA and RNA (Lalchhandama, K. 2016).

Fluorescein

It is a fluorescent probe that is generally used in forensics to detect latent bloodstain even if it is diluted to 1:1000; in the field of ophthalmology, fluorescein sodium employed as a diagnostic tool. During open-heart surgery, diluted fluorescein has been used to detect or localize ventricular septal defects (Budowle et al., 2000 and Mathew et al., 2014).

Table 1. Fluorescent Probes used in the Detection of Different Chemicals in the Body (Leung et al., 2013, Lee et al., 2018 and Wang et al., 2019).

Fluorescent Probe	Detects
Azulene chemo dosimeter imaging fluorescent probe	Reactive Oxygen and Nitrogen species
Mito-HT fluorescent probe	Hydrogen peroxide (H_2S) in live cells
Superior fluorescent probes	Cardiolipin
Biotinylated fluorescent probe	Blood glucose
Mag-fura-2(based on APTRA binding site)	Magnesium

Green Fluorescent Protein	Onset and termination of calcium signalling
DNZ-Az Fluorescent probe	Hydrogen sulphide

Fluorescence Detection and Quantitation

Detection

to identify the fluorescence, some tools and instruments have been developed, and each and every instrument has unique features and distinct properties for various experimental methods.

Every fluorescence detection instrument has some basic components: (1) A lighting source, (2) A fluorophore, (3) Filters to isolate specific wavelengths, (4) A detector (electronic device) that detects incoming signal or records the output.

Common fluorescence detection instrumentation includes: (a) Fluorescent microscopes: used working principle of fluorescence (b) Fluorescence scanners: 2-dimensional detection of fluors in samples (c) Spectrofluorometers and microplate readers: typical fluorescence measurement in specimens (d) Flow cytometers: analysis and detection of fluorescence in the sample population.

QuantitationFluorescent Signal Quantification has become commonly used in all the fluorescent fields and got extensive attention towards the measurement of a large number of parameters, including (a) rate of movement of cells or intracellular constituents (b) protein, DNA or RNA amount in the sample (c) protein, DNA or RNA sequencing (d) cell number (e) enzyme function/activity (d) applicability.

Fluorescence quantitation requires particular software, and according to the demand of experimental procedures, the instrumentation is calibrated by using a variety of fluorescent standards.

Fluorescent Labelling

Fluorescent labelling is that method in which a molecule as nucleic acid or protein is covalently bound to the fluorophore. This is generally done by utilizing a reactive derivative of the fluorophore that selectively attaches to a functional group existing within the target molecule. The extensively utilized labelled molecules used as specific probes are antibodies for the detection of a target. Fluorescent labelling enables sensitive and quantitative measurements that can pertain to a wide variety of detection systems.

In 1942 Fluorescent labelling with a synthetic fluorophore was first documented when fluorescein isothiocyanate (FITC)-labelled anti-pneumococcal antibodies were attained (Martynov et al., 2016).

Detection of Chemicals in Blood

Detection of Hydrogen Sulphide (H_2S)

Hydrogen sulfide is a vital gasotransmitter that plays a significant role in many physiological processes in the gastrointestinal, cardiovascular, nervous, endocrine, immune system (Lin et al., 2019 and Peng et al., 2011). 10-100 μM is the range of H2S concentration present in the blood. (Whitfield et al., 2008 and Xuan et al., 2012). Altered hydrogen sulfide levels are associated with numerous diseases, including Downs Syndrome, gastrointestinal, liver cirrhosis, chronic kidney diseases, etc. (Qian et al., 2012). Various techniques used in the detection of H_2S comprise electrochemical, gas chromatography and electrochemical assay (Olson, K. R. 2012). These techniques do not enable fast, sensitive, and actual-time determination. Fluorescence is a more sensitive and valid method for the identification of H_2S (Chen et al., 2013). Fluorescent probes are of tremendous importance in this regard due to their elevated selectivity and sensitivity, simple management, increased emission rates and fewer toxicity levels to cells and tissues (Ueno et al., 2011, Gao et al., 2017 and Yang et al., 2018). Hydrogen sulfide is present in extracellular matrix and plasma as 20% H_2S, 80% HS- ion and in inconsequential amounts that are S-2, collectively termed as sulfides (Olson et al., 2009). These sulfides are measured and detected in body fluids using bulk sampling, but it is cumbersome for clinical use. This can be resolved by miniaturization of detection and measuring systems whose multiple advantages are encompassing better and precise assessment of volatile substances that are short-lived, reduced sample and reagent consumption. Attempts have been made to detect hydrogen sulfide in biosamples out miniaturization by conjugating nanoparticle with a fluorophore, followed by incubation of PTFE tube with droplet samples, and that is determined by the real-time PCR instrument (Karunya et al., 2019).

Dansyl is the most common used fluorophore and has favorable characteristics such as powerful fluorescence and extended emission wavelength. Peng et al. constructed a sulfide-sensitive agent using the above-mentioned fluorophore by the

decrease of an azido group by hydrogen sulfide. DNS-Az itself is nonfluorescent. Whereas by the improvement of hydrogen sulfide, the DNS-Az solution showed a powerful fluorescence (Peng et al., 2011)

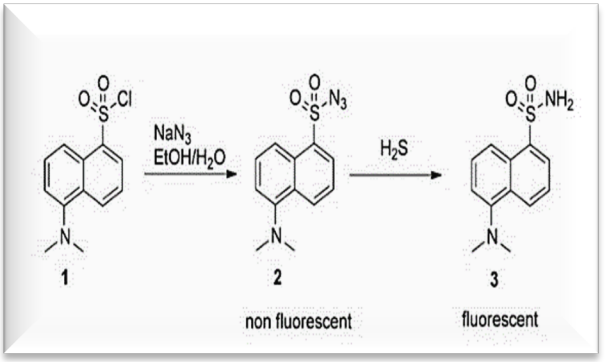

Figure 1: Schematic Manifestation of the Synthesis of DNS-Az (fluorescence) probe (Peng et al., 2011).

Detection of H_2S In Blood by Using Fluorescent Probes

Karyuna et al. (2019) reported a newly efficient microfluidic method using a Dansyl-azide Fluorescence (FL) based probe for rapid detection of chemical sulphide in blood plasma. The attained results exhibited that with the boost in exogenous sulphide concentration in plasma, FL intensity decreases that showed the strong relations between proteins and sulphide existing in plasma. The studies determined the impact of different parameters such as pH, incubation time, mixing, probe concentration etc., on FL intensity. Results revealed that the FL assay required a mixing time of 2 min, an incubation time of 5 min, a pH of 7.1 and also the linear correlation obtained value was (with $R2 \geq 0.95$), which was close enough with the previously reported method. 70 µM to 125 µM range of endogenous sulfide levels in the plasma of healthy individuals were observed. A microfluidic device with a DNS-Az probe for the fast and quantitative estimation of H_2S in plasma was reported according to the respective study. According to the study, the availability of the microfluidic device will help in establishing the role of H_2S in health and disease in, near future.

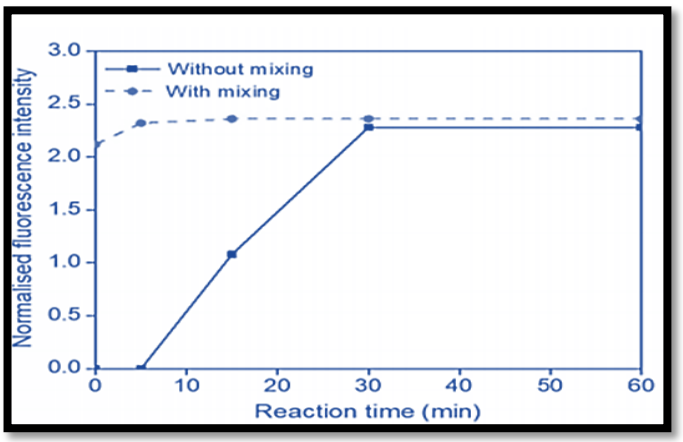

Figure 2: Graph showing the effect of incubation time and mixing on FL intensity (sulphide concentration (100µM) in 1.0mL of Phosphate Buffer Saline PBS, in one of the sample, (by using a vortex mixer for the 30s) sulphide and probe were wholly mixed externally, while in the other, sulphide and probe were added without mixing (Karunya et al., 2019).

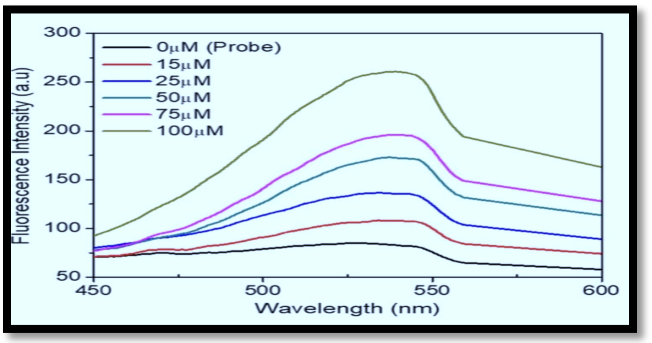

Figure 3: Fluorescence spectrum variation concerning the concentration of sulphide in PBS (Karunya et al., 2019).

Peng et al. (2011) used a novel fluorescence chemo probe DNS-Az for recognition of hydrogen sulfide in various mediums as blood serum, whole blood and solutions that are aqueous. C57BL6/J mouse model was used while detecting H2S in blood, and the result obtained 31.9 ± 9.4 μM that was extremely closely related to the formerly documented serum level of hydrogen sulfide. For the future perspective, this method of detection for H_2S while using the DNS-Az probe is a highly productive, selective, low-cost detection method as reliable for further research purposes.

Yang et al. (2018), by modifying ICT-based two-photon fluorescent dye (4-hydroxy-1, 8-naphthalimide) with cyanate (RO-CN), developed a TP fluorescent probe as an H_2S specific recognition unit. Results illustrated that a detection limit of 0.24 μM vast linear range of 1-10 μM as well as a sharp response time (< 90 s) was attained while detecting H_2S by using the respective probe. The probe showed increased selectivity of biologically active compounds and other cations/anions and also showed better accuracy in detecting H_2S. Studies confirmed that the probe has the capacity of TP imaging for recognizing H_2S in the living cells that enhance its usefulness for practical applications in physiological systems.

Qian et al. (2012) synthesized BODIPY-based probe SFP-3 for the analysis of H_2S depicted by varied techniques such as Nuclear Magnetic Resonance, spectroscopy, and mass spectroscopy. Outcomes indicated that the average concentration of sulfide was 56.0 ± 2.5 μM in the respective model of blood plasma that was very close to the formerly published reports on concentrations of sulfide in blood plasma. The synthesized probe showed elevated potential for the quick and real-time detection of sulfide in different sorts of physiological samples. In future, much more efforts are required for synthesizing accurate and particular fluorescent probes for optical imaging and surveys of H_2S metabolism that are mechanistic in biological systems.

Detection of Iron in Blood

Iron is a trace element that is vital at all times so that the body can work properly and performs its physiological functions. It serves as a cofactor for many enzymes and proteins that are required for oxygen transport and energy metabolism, as well as for many other vital processes (Long et al., 2014). The abnormal iron levels may result in serious diseases as anaemia caused due to deficiency of iron. The iron is bound to the transferrin that acts as iron transport protein in blood serum, in ferric (Fe3+) form. For the detection of Fe3+ in blood serum, several techniques, including colorimetry, spectrophotometry, resonance ionization isotope dilution mass spectrometry, atomic absorption spectrometry, and inductively coupled plasma mass spectrometry (ICP-MS), have already been documented. Whereas fluorescence sensing has been adopted due to its various significant features that are high sensitivity, fast response time and simplicity of technique. Till now, many different fluorescent probes have been designed that help in detection of iron (Fe3+), but only limited probes are used for the detection and identification of iron in blood serum, such as NBD-DFO and calcein etc.

Detection of Iron in Blood by Using Fluorescent Probes

Long et al. (2014) designed a versatile ratio-metric fluorescent probe 1 for the detection of iron in the blood.

The reaction mechanism involved here is acetal deprotection (Fe^{+3} mediated). Some of the significant characteristics of the probe that were documented by the respective study were high emission ratio variation, broad emission shift, elevated sensitivity, selectivity. Findings showed the ratio-metric response of the probe, with the fluorescence spectra illustrating considerable redshift and the emission ratio value up to 132nm and I522/I390, respectively, with the expansion of 23632 folds. The probe sensitivity was quantified, and the detection limit found out to be 0.12M. Also, the probe showed elevated selectivity for Fe^{+3} as compared to other related metal ions.

Yang et al. (2019) evaluated the performance of a novel TP and turned on a NIR probe based on intramolecular charge transfer for Fe2+ quantification. Findings demonstrated the rapid response of 15min and the detection limit of 4.5μM with elevated selectivity and sensitivity while inspecting the Fe2+. The recent study documented fluorescent probe's practical application in evaluating iron with Two photos microscopic FL imaging, which was not done before. So, the probe was highly recommendable for labile Fe2+ detection using TP detection.

Detection of Glucose in Blood

Blood glucose concentration is the concentration of glucose in the blood. Glucose is very crucial as it generates energy that assists the body in its daily functions. The blood sugar levels should be in a safe range to minimize the risk of diseases as heart attack and diabetes that are evolving very promptly (Lee et al., 2018). An unprecedented proportion of blood glucose can be associated with numerous ailments such as hypoglycemia and diabetes. Hence, the improvement of an immediate, selective, and sensitive analytical method for the measurement of glucose concentration is remarkably beneficial in the diagnosis and crucial scientific research.

Detection of Blood Glucose by Using Fluorescent Probes

Lee et al. (2018) reported an avidin protein encapsulated fluorescent probe. Studies demonstrated that the probe could yield specific fluorescence, which is achieved by non-specific dye-protein interaction blockage in blood serum. The strategy used stood very successful in deducing the quantity of glucose concentration in blood serum.

Sun et al. (2015) proposed that among all glucose-sensing methods that are based on fluorescence, turn-on chemical probes (fluorescent) are the most beneficial contenders due to their properties as raised sensitivity, simple and quick operation, and dynamic range.

Hong et al. (2010) reported that fluorescent probes work efficiently in aqueous buffers, but it faces challenges in protein-rich human blood as it is hidden by non-specific fluorescence. The dilemma occurs as the fluorescent dyes bind to the blood proteins like globulin, albumin modifying the fluorescent properties resulting in the incorrect clinical diagnosis and overestimation of glucose concentrations leading to hypoglycemia or hyperglycemia.

Zeng et al. (2016) reported the evolution of a new hydrogen peroxide responsive probe BTSBD-B(OH) 2 for deducing blood glucose concentration in protein-rich samples in coupling assay with glucose oxidase.

Wu et al. (2016) reported FFEPC to obstruct non-specific interaction of the blood proteins with the probe.

Dundas et al. (2011) reported that through the FPEPC approach, when the avoiding protein combines with biotinylated fluorescent probes, it can obstruct the binding of dye that is non-specific with no target blood proteins to prevent signals that are non-specific. Biotin and avidin have strong binding described by a Kd value of 10-15M, and the consequent complex is stable in the presence of denaturing agents and high temperature and pH levels. The issue of weak fluorescence in an aqueous solution of SBD dye was resolved by conjugating a biotinylated fluorescent probe to avidin that ultimately increases fluorescence, also improving the detection sensitivity.

Further reported that the calculated blood glucose levels by BTSBD-B(OH)2 – conjugate with avidin that was 7.18 and 5.63mM that lies in the normal blood glucose concentration in human. Thus, unfolding our avidin conjugated probe reliance. Concentration levels of glucose 32.83 and 26.46 mM were obtained while detecting TSBD-B(OH)2 with two blood serum samples. More accurate results with improved sensitivity of fluorescent probe can be obtained through the FPEPC approach (Lee et al., 2018).

Detection of Glutathione in Blood

Glutathione is a thiol of prime importance present

mainly in the form of GSH in the body. Glutathione is a crucial antioxidant within the body (Li et al., 2020). Imbalance of GSH leads to a number of pathologies including Parkinson's disease, cystic fibrosis, HIV, cancer. Therefore, its quantification in the blood is of utmost importance. Studies reported various methods for the quantification of glutathione in the blood namely electrochemistry, mass spectrometry, liquid chromatography. Spectrofluorimetric methods have gained more importance in this regard due to their high sensitivity.

Detection of Glutathione by Using Fluorescent Probes

Li et al. designed SN-2 stimulated and Intramolecular charge transfer based novel probe (fluorescent) for glutathione quantification. CBF was the probe used due to its elevated value of quantum yield equivalent to 0.85, increased bioavailability and TP absorption spectrum.

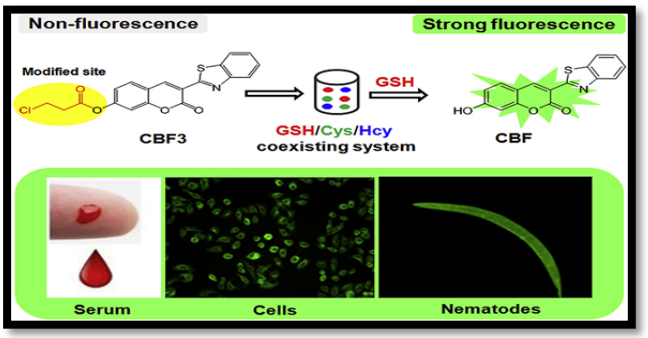

Figure 4: GSH detection in the serum sample, living cell the s and nematodes with CBF probe (Li et al.,2020).

CBF3 came out to be more selective and sensitive for detection of GSH as compared to the other three probes CBF1, CBF2 and CBF4, which did not show an appreciable selectivity for glutathione. As reported, GSH exists together with Cysteine and Homocysteine in the living system at much higher levels as compared to them. This probe was found to be more selective for glutathione, and its recognition for GSH remained unaffected even in the presence of Cys and Hcy. Studies showed this by the correlation $R2 > 0.99$, $p < 0.0001$ between the data obtained from the glutathione and other related groups containing it, the detection limit for this probe was 9.2nM. Thus, we can easily use this probe for glutathione detection in serum samples, nematodes and living cells model. This approach can also be used for designing the other fluorescent probes for GSH detection (Li et al., 2020).

Lui et al. (2018) reported a heptamethinine cyanine probe (DNIR) with emission and excitation (NIR)excitation 832nm and 804nm, respectively, for the reduced and oxidized glutathione detection (GSH and GSSG) in blood. The limit for detection found out to be 87.2 nM. According to the studies conducted, it was found out that an increase in the GSH concentration, FL intensity also increased. The reaction mechanism involves the SN2 reaction of a thiol group to azoethyl ether to detect glutathione and the ratio of its reduced and oxidized form in the presence of NADPH and GR. The reaction component and quencher (nitro-azo-ether group) goes through the cleavage by thiols and turn-on fluorescence emission. Studies showed this probe had increased selectivity for glutathione and a fast response time of 3 min. The studies performed showed that this probe could be used for the efficacious monitoring of glutathione in the blood and the ratios of its reduced and oxidized forms.

Conclusion

In conclusion, an overview of the fluorescent probes, their certain types and applications are discussed. The methods for the chemical's identification and quantification in the blood are discussed to give an insight into the appropriate applications of fluorescent probes. In this review article, certain newly synthesized fluorescent probes have discussed that help in the detection and quantification of certain chemicals like hydrogen sulphide, iron, blood glucose, glutathione etc.

Functional probes in this regard have emerged as absolute necessary tools in modern biology as they deliver us vital information regarding the amount and emplacement of molecules of interest.

Abbreviations Used

GFP: Green Fluorescent Protein
QDs: Quantum Dots
DNS-AZ: Dansyl azide
APTRA: Alpha aminophenol-N, N,0-triacetic acid
FITC: Fluorescein isothiocyanate
PTFE: Polytetrafluoroethylene
PCR: Polymerase Chain Reaction
FL: Fluorescence
PBS: Phosphate buffer saline
TP: Two photon
ICT: Intramolecular charge transfer
NMR: Nuclear Magnetic Resonance
BODIPY: Boron dipyrromethene
SPF3: Sirtuin fluorescence probe
ICP-MS: Inductively coupled plasma mass spectrometry
FL-DFO: Fluorescein des-ferrioxamine
NBD-DFO: 7-Nitrobenz-2-oxa- 1,3-diazole des-ferrioxamine
NIR: Near infrared
TPM: Two-Photon Microscopy
FFEPC: Fluorescent probe encapsulated in protein cavity
GSH: Reduced Glutathione
HIV: Human Immunodeficiency Virus
SN-2: Substitution Nucleophilic Bimolecular
CBF: 3-Benzothiazolyl-7-hydroxycoumarin
Cys: Cysteine
Hcy: Homocysteine
GSSG: Oxidized Glutathione
GR: Glutathione Reductase
NADPH: Nicotinamide Adenine Dinucleotide Phosphate

References

Budowle, B., Leggitt, J., Defenbaugh, D., Keys, K., & Malkiewicz, S. (2000). The Presumptive Reagent Fluorescein for Detection of Dilute Bloodstains and Subsequent STR Typing of Recovered DNA, Journal of Forensic Sciences, 45, (5)1090-1092.

Chandan, H. R., Schiffman, J. D., & Balakrishna, R. G. (2018). Quantum dots as fluorescent probes: Synthesis, surface chemistry, energy transfer mechanisms, and applications. Sensors and Actuators B: Chemical, 258, 1191–1214.

Chen, B., Li, W., Lv, C., Zhao, M., Jin, H., Jin, H., & Tang, X. (2013). A fluorescent probe for highly selective and sensitive detection of hydrogen sulfide in living cells and cardiac tissues. The Analyst, 138(3), 946–951.

Gao, B., Cui, L., Pan, Y., Xue, M., Zhu, B., Zhang, G., & Dong, C. (2017). A highly selective fluorescent probe based on Michael addition for fast detection of hydrogen sulfide. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 173, 457–461.

Karunya, R., Jayaprakash, K. S., Gaikwad, R., Sajeesh, P., Ramshad, K., Muraleedharan, K. M., & Sen, A. K. (2019). Rapid measurement of hydrogen sulphide in human blood plasma using a microfluidic method. Scientific Reports, 9(1).

Kumar, A., & Pal, D. (2016). Green Fluorescent Protein and Their Applications in Advance Research, Journal of Research in Engineering and Applied Sciences.

Lalchhandama, K. (2016). The making of modern biotechnology: how ethidium bromide made fame. Science Vision 16 (1), 27-36.

Lee, F. H., Chew, C. Y., Hwu, J. R., & Tan, K. T. (2018). A biotinylated fluorescent probe for the specific and quantitative determination of blood glucose. Journal of the Chinese Chemical Society, 66, 114–118.

Leung, C. W. T., Hong, Y., Hanske, J., Zhao, E., Chen, S., Pletneva, E. V., & Tang, B. Z. (2013). Superior Fluorescent Probe for Detection of Cardiolipin. Analytical Chemistry, *86*(2), 1263–1268.

Li, H., Yang, Y., Qi, X., Zhou, X., Ren, W. X., Deng, M., & Teichmann, A. T. (2020). Design and Applications of a Novel Fluorescent Probe for Detecting Glutathione in Biological Samples. Analytica Chimica Acta.

Lin, X., Lu, X., Zhou, J., Ren, H., Dong, X., Zhao, W., & Chen, Z. (2019). An instantaneous fluorescent probe for the specific detection of H2S. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.

Liu, C., Qi, F., Wen, F., Long, L., Liu, A., & Yang, R. (2018). Fluorescence detection of glutathione and oxidized glutathione in blood with a NIR-excitable cyanine probe. Methods and Applications in Fluorescence, *6*(2), 024001.

Long, L., Zhou, L., Wang, L., Meng, S., Gong, A., & Zhang, C. (2014). A ratiometric fluorescent probe for iron (III) and its application for detection of iron (III) in human blood serum. Analytica Chimica Acta, 812, 145–151.

Martynov, V. I., Pakhomov, A. A., Popova, N. V., Deyev, I. E., Petrenko, A. G. (2016). Synthetic fluorophores for visualizing biomolecules in living systems, 08, 33-46.

Masilamani, V., Al-Zhrani, K., Al-Salhi, M., Al-Diab, A., & Al-Ageily, M. (2004). Cancer diagnosis by autofluorescence of blood components. Journal of Luminescence, *109*(3-4), 143–154.

Matea, C., Mocan, T., Tabaran, F., Pop, T., Mosteanu, O., Puia, C., & Mocan, L. (2017). Quantum dots in imaging, drug delivery and sensor applications. International Journal of Nanomedicine, *12*, 5421–5431.

Mathew, T., Kundan, S., Abdulsamad, M. I., Menon, S., Dharan, B. S., & Jayakumar, K. (2014). Multiple Muscular Ventricular Septal Defects: Use of Fluorescein Dye to Identify Residual Defects. The Annals of Thoracic Surgery, *97*(1), 27–28.

Olson, K. R. (2009). Is hydrogen sulfide a circulating "gasotransmitter" in vertebrate blood? Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1787(7), 856–863.

Olson, K. R. (2012). A Practical Look at the Chemistry and Biology of Hydrogen Sulfide. Antioxidants & Redox Signaling, 17(1), 32–44.

Peng, H., Cheng, Y., Dai, C., King, A. L., Predmore, B. L., Lefer, D. J., & Wang, B. (2011). A Fluorescent Probe for Fast and Quantitative Detection of Hydrogen Sulfide in Blood. Angewandte Chemie International Edition, 50(41), 9672–9675.

Qian, Y., Zhang, L., Ding, S., Deng, X., He, C., Zheng, X. E., & Zhao, J. (2012). A fluorescent probe for rapid detection of hydrogen sulfide in blood plasma and brain tissues in mice. Chemical Science, 3(10), 2920.

Sun, X., & James, T. D. (2015). Glucose Sensing in Supramolecular Chemistry. Chemical Reviews, 115(15), 8001–8037.

Ueno, T., Nagano, T. Fluorescent probes for sensing and imaging. Nat. Methods 2011, 8, 642–645

Wang, C., Wang, Y., Wang, G., Huang, C., & Jia, N. (2019) A new mitochondria-targeting fluorescent probe for ratiometric detection of H2O2 in live cells, Analytica Chimica Acta, S0003-2670(19), 31368-6

Whitfield, N. L., Kreimier, E. L., Verdial, F. C., Skovgaard, N., & Olson, K. R. (2008). Reappraisal of H2S/sulfide concentration in vertebrate blood and its potential significance in ischemic preconditioning and vascular signalling. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, *294*(6), 1930–1937.

Xuan, W., Sheng, C., Cao, Y., He, W., & Wang, W. (2012). Fluorescent Probes for the Detection of Hydrogen Sulfide in Biological Systems. Angewandte Chemie International Edition, 51(10), 2282–2284.

Yang, G., Zhang, J., Zhu, S., Wang, Y., Feng, X., Yan, M., & Yu, J. (2018). Fast response and highly selective detection of hydrogen sulfide with a ratiometric two-photon fluorescent probe and its application for bioimaging. Sensors and Actuators B: Chemical, 261, 51–57

Yang, X., Wang, Y., Liu, R., Zhang, Y., Tang, J., Yang, E., & Ye, Y. (2019). A novel ICT-based two-photon and NIR fluorescent probe for labile Fe2+ detection and cell imaging in living cells. Sensors and Actuators B: Chemical.

Zeng, Y. S., Gao, R. C., Wu, T. W., Cho, C., & Tan, K. T. (2016). Fluorescent Probe Encapsulated in SNAP-Tag Protein Cavity to Eliminate Non-specific Fluorescence and Increase Detection Sensitivity. Bioconjugate Chemistry, 27(8), 1872–1879.