Chapter-1

Introduction

1. Introduction

1.1. Introduction to Fluorescence

The absorption of light (photons) by a group of molecules induces the promotion of electrons from their singlet ground electronic level (S_0) to an excited state S_n ($n > l$).[1] The molecule in the excited state then returns to the ground state (S_0) in two steps:

I. The molecule at S_n moves to the lowest excited state (S_1) by releasing some of its energy to the surrounding environment, a process known as **internal conversion**.

II. From the excited state S_1, the molecule reaches the ground state S_0 through various competing processes:

• Emission of a photon as radiation, known as **fluorescence**.

• Dissipation of absorbed energy as **non-radiative heat**.

• Transfer of energy to nearby molecules through **collisional quenching** and/or **Forster Resonance Energy Transfer (FRET)**.

• Occasional transition of electrons from the excited singlet state (S_1) to the excited triplet state (T_1) through **inter-system crossing**. When the molecule returns to the ground state (S_0) from a triplet excited state (T_1) by emitting a photon as radiation, it is called **phosphorescence**.

In molecular spectroscopy, these transitions between the electronic states of a molecule are effectively illustrated using a Jablonski diagram (see figure 1.1).[2]

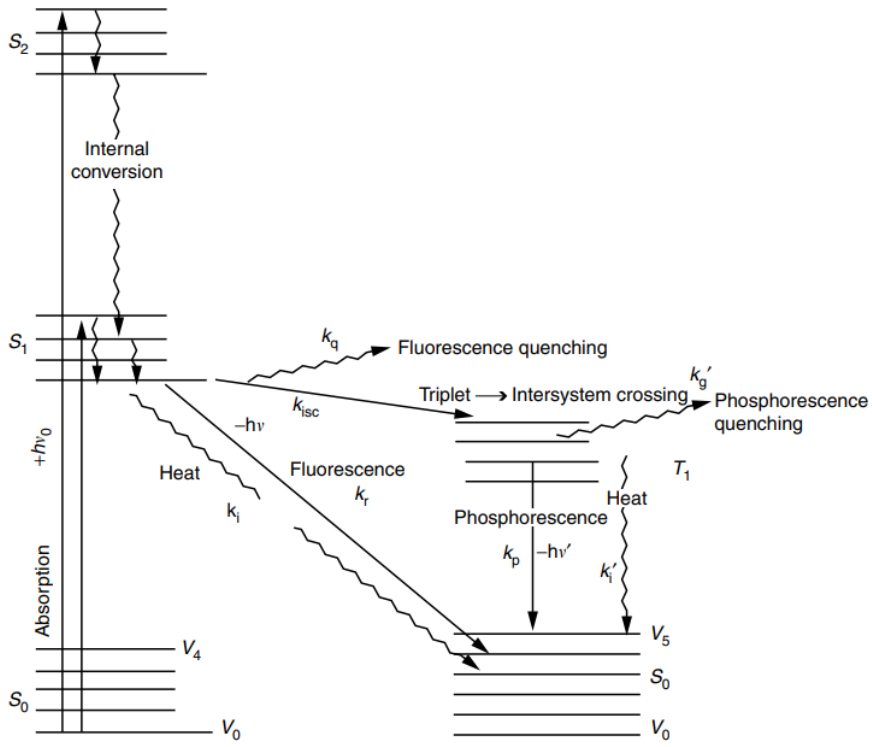

Figure 1.1. Jablonski (electronic transitions) diagram. Adapted from Ref. [2].

1.2. Fluorescent Dyes

Fluorescent dyes are organic molecules with a donor-pi-acceptor (D-π-A) electronic system. When irradiated with high-power light of a specific wavelength, they emit coloured light of a higher wavelength and lower energy. The wavelength of light at which a fluorescent dye is excited is called its absorption or excitation peak (λ_{abs}), while, the wavelength at which it emits light is referred to as its emission peak (λ_{em}). The difference between λ_{abs} and λ_{em} is called the Stokes shift named after scientist George Stokes, who identified and named the phenomenon of fluorescence.[3]

1.2.1. A Brief History of Fluorescent Dyes

The advent of fluorescent dyes started with the serendipitous creation of the synthetic dye mauvine by Perkin in 1856. Incessant research on the development of new synthetic dyes continued for the next 70 years until the discovery of fluorescein isothiocyanate (FITC) in 1942

which sparked the field of fluorescence microscopy. The development of fluorescent dyes saw a surge in the 1960s and 1970s with the formation of various rhodamine and boron dipyrromethene (BODIPY) dyes. The 1990s marked the definitive arrival of commercial collections of fluorophores, including the CyDye, Alexa Fluor, and ATTO series, all featuring significantly improved properties. Since 2000, Biotium has introduced an extensive series of highly water-soluble fluorescent CF® dyes specifically designed for labeling proteins and nucleic acids (figure 1.2).[4]

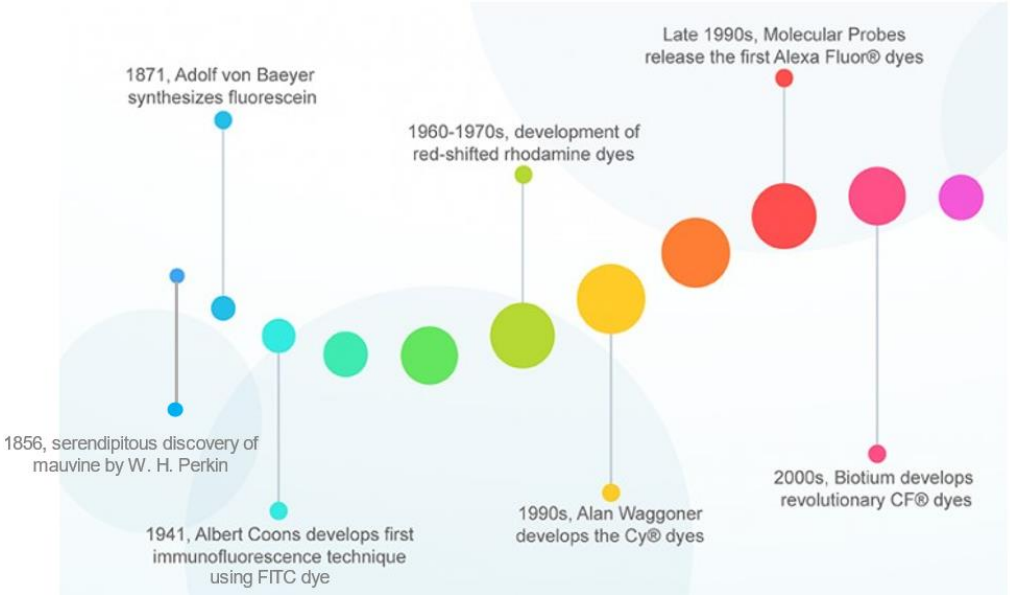

Figure 1.2. The timeline illustrating the emergence and steady advancement of fluorescent dyes over the years. Adapted from Ref. [4].

1.2.2. Role of Fluorescent Dyes in Fluorescence Imaging

Fluorescent dyes are extensively utilized in the imaging of cell organelles, detection of biomolecules, and labelling or tagging of antibody conjugates or peptides. They can also be used to measure various biochemicals produced in response to pathological conditions and to track molecular processes inside living cells that were previously difficult to study. Because of these advantages, fluorescent dyes are widely used in a variety of research and therapeutic

applications.[5] The following are key examples of classical fluorescent dyes used in cell imaging (refer to figure 1.3). Ethidium bromide serves as a nuclear stain in molecular biology, intercalating with double-stranded DNA to emit pink light. Another crucial nuclear stain is 4',6'-diamino-2-phenylindole (DAPI), extensively used in fluorescence microscopy, and provides a marker for membrane viability as it passes through the membrane less efficiently in live cells. Hoechst dyes are a group of nuclear stains that can easily permeate live membranes and are therefore employed to stain live cells. Cellular proteins are stained using hydrophilic fluorescein isothiocyanate (FITC) dyes.[6] Coumarin-6 is a hydrophobic blue-green spectrum dye, primarily used for the labeling and visualization of polymeric nanoparticles in cell cytoplasm. ATTO-550 is an advanced yellow light-emitting dye used for tagging DNA. MitoTracker orange and tetramethylrhodamine (TMR) dyes are popularly used for the localization of mitochondria inside cells. Additionally, to measure changes in the mitochondrial membrane potential, JC1 dyes are mostly employed. Further, the Alexa Fluor class of dyes can be used for staining a variety of cellular parts; for example, Alexa Fluor 350 is used for imaging of the endoplasmic reticulum.

1.2.3. Fluorophores and Auxochromes

The chemical structure of such fluorescent dyes is comprised of two basic units: (a) fluorophores (also known as fluorochromes) and (b) auxochromes/auxofluores. Fluorophore (or fluorochrome) is that portion of the chemical scaffold that is responsible for the emission of light from dyes as it contains an extended conjugated system and combined aromatic groups. These fluorophores can be broadly classified into various chemical scaffolds (refer to figure 1.4), such as coumarins, cyanines, xanthenes, fluorescein, rhodamine, benzothiazoles, dipyrromethenes, naphthalimides, nitrobenzoxadiazoles, carboxamidines, and benzamidines.[6] Fluorophores are functionalized with non-emissive functional groups known as auxochromes, which contain one or more lone pairs of electrons and play a great role in modulating the

photophysical as well as physicochemical properties of these fluorophores. Small changes in auxochromes can fine-tune the absorption and emission maxima of a fluorophore, its quantum yield (Φ_f), brightness, photostability, molar absorptivity (ε), aqueous solubility, and membrane permeability.

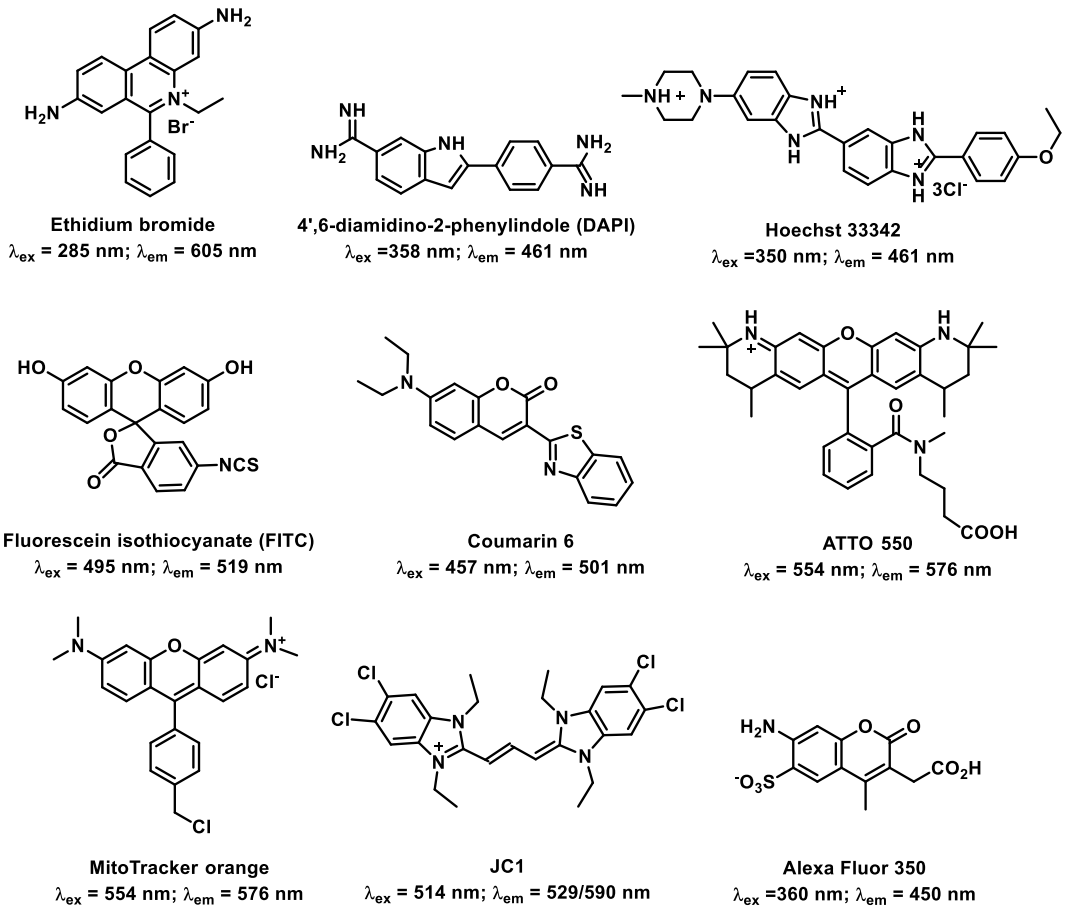

Figure 1.3. Classical fluorescent dyes used for imaging of cells and various cell organelles.

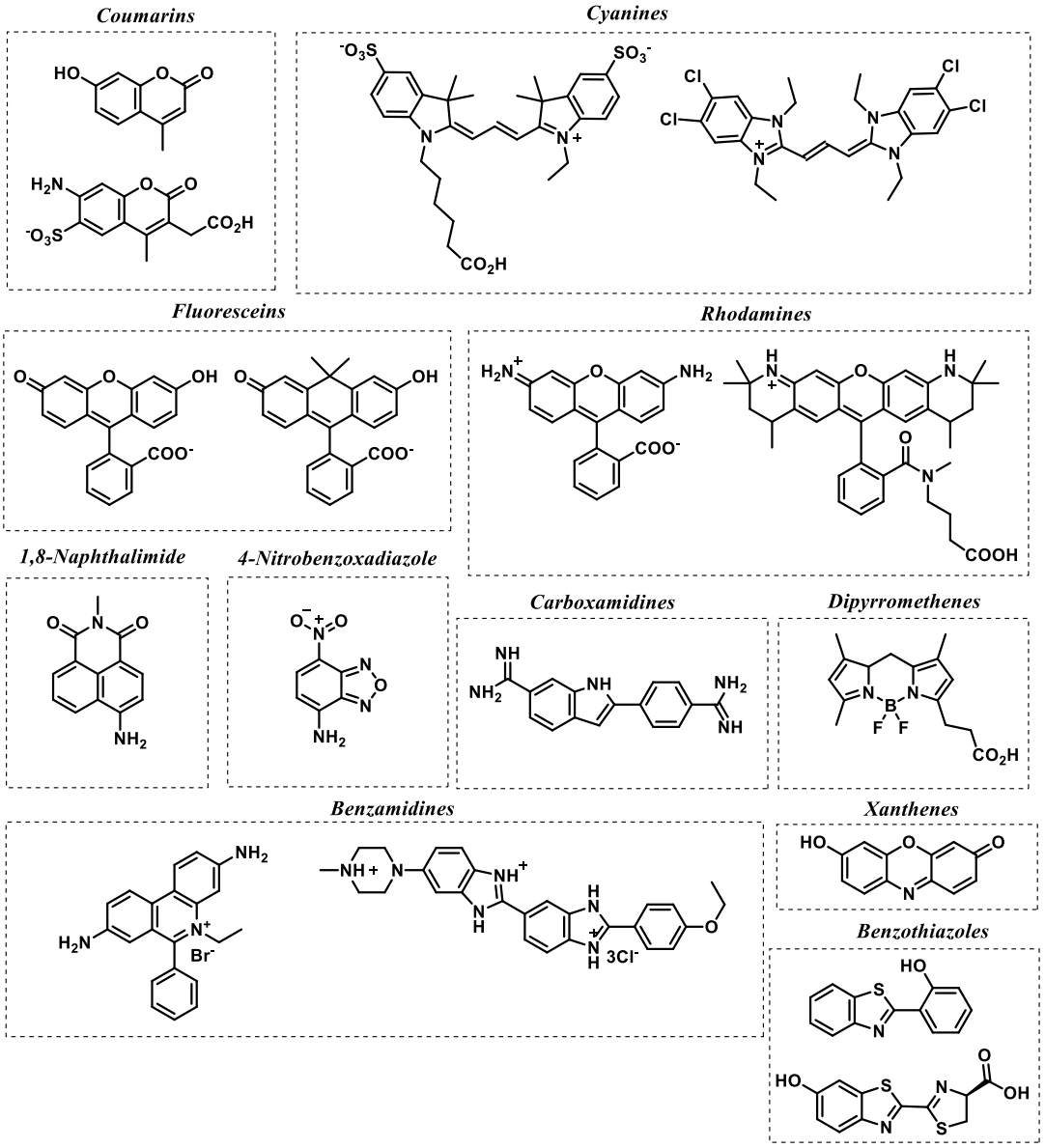

Figure 1.4. Examples of various classes of fluorophores found in dyes used for bioimaging.

1.2.4. Properties of an Ideal Fluorophore

An ideal fluorophore must exhibit the following properties: (a) Φ_f equal to 1 to ensure excellent brightness, (b) modulated to any wavelength to ensure deep tissue penetration, (c) absolute biosafety with minimal photodamage to living cells, (d) indefinite photostability under any range of illumination, and (e) functional in all biological systems. Such types of ideal fluorophores are practically non-existent, so fluorophore developers aim to achieve the following: (a) increase ε and Φ_f to maximize molecular brightness, (b) decrease the chances of

intersystem crossing to prevent the generation of singlet oxygen and other toxic reactive oxygen species (ROS), (c) chemical modification of the fluorophore to endow them with desirable properties, such as aqueous solubility, cell permeability, and resistance to photobleaching (degradation of fluorophore by any biochemical reaction).[6]

1.2.5. Role of Auxochromes in Modulating Fluorogenic and Physicochemical Properties of Various Fluorophores.

The properties of desirable fluorophores can be achieved by making precise alterations to the auxochrome units.[6,7] Let us consider the example of coumarins to comprehend the impact of auxochromes on the fluorogenic properties of the fluorescent scaffold (figure 1.5). Upon replacing the 7th position hydroxyl (-OH) group of the naturally occurring fluorescent dye β-methylumbelliferone (**1a**, λ_{abs}= 320 nm, λ_{em}= 450 nm, ϕ_f = 0.356) with an amino (-NH_2) group, the resulting molecule **1b** (λ_{abs}= 342 nm, λ_{em}= 441 nm, ϕ_f = 0.50) demonstrated a substantial increase in Φf due to enhanced electron donation by amines. It was expected that further enhancement of electron donation might lead to a higher quantum yield. Consequently, dimethylamino **(1c)** and diethylamino **(1d)** derivatives of 7-aminocoumarins were synthesized. Despite displaying a desirable red-shift in their absorption and emission maxima, these derivatives (**1c,** λ_{abs}= 372 nm, λ_{em}= 470 nm, ϕ_f = 0.19 and **1d,** λ_{abs}= 381 nm, λ_{em}= 468 nm, ϕ_f = 0.06) experienced a significant decrease in Φf with an increase in the degree of alkyl substitution. After numerous experiments, it was conclusively determined that the sudden decrease in Φf is caused by a phenomenon called twisted intramolecular charge transfer (TICT). In this process, fluorophores, **1c** and **1d** absorb a photon to give their corresponding excited states (**1c*** and **1d***; figure 1.6). This is followed by an electron transfer from the nitrogen atom to the coumarin ring system, accompanied by twisting of the C_{aryl}-N single bond. TICT is favoured energetically in 7-dialkylaminocoumarin dyes due to their lower ionization

potential. The TICT forms relax, causing a rapid non-radiative energy decay of the excited state without emitting any light. These diradical intermediates (**1c**$_{TICT}$ and **1d**$_{TICT}$) may also undergo irreversible photobleaching, which leads them to have poor quantum efficiency, shorter fluorescence lifetime and reduced photostability. After several years of research, Grimm *et al.* discovered a solution to this issue, by replacing the *N,N*-dialkyl groups with sterically hindered azacyclic rings, spanning from azetidine to azepane). Among these, 7-azetidinocoumarin (**1e**, λ_{abs}= 354 nm, λ_{em}= 471 nm, ϕ_f = 0.96) successfully restricted the occurrence of TICT and exhibited enhanced fluorogenic properties. Additionally, Liu *et al.* observed that 7-aziridinocoumarin (**1f**, λ_{abs}= 325 nm, λ_{em}= 439 nm, ϕ_f = 0.88) effectively suppresses TICT similar to its azetidinylated counterpart, **1e,** due to higher ring strain while showcasing improved brightness, superior photostability, and enlarged Stokes shifts. Despite their excellent fluorogenic properties, the azacyclic ring-based coumarin dyes faced severe solubility issues. To address this, Zhou *et al.* postulated incorporating spirooxetane/spiroazetidine rings as the donor moiety instead of azetidine in coumarin scaffolds. The azetidine half of the spiroazetidine ring helps to suppress TICT and afford bright fluorescence, while, the second ring of the spirocycle helps adjust the balance between the solubility and permeability of the coumarin core. The steric congestion also contributes to maintaining photostability.[8] The resulting derivatives **1g** (λ_{abs}= 352 nm, λ_{em}= 463 nm, ϕ_f = 0.96, LYSA= 38 μg/mL) and **1h** (λ_{abs}= 355 nm, λ_{em}= 461 nm, ϕ_f = 0.97, LYSA= 340 μg/mL) demonstrated significantly improved solubility compared to compound **1e** (LYSA< 1 μg/mL). Despite possessing all the characteristic features of an ideal fluorophore, the higher cost of reaction substrates for constructing these molecules (**1g** and **1h**) and lack of synthetic feasibility present substantial challenges to their development. A more economical alternative for highly soluble coumarin fluorophores was recently presented by Indurthi and the group,[9] where they replaced spirooxetane with azaspiroketals. The resulting molecule (**1i**) exhibited excellent solubility

(LYSA = 695 μg/mL), enhanced molar absorptivity and phenomenal brightness. However, **1i** suffered from a severe loss of quantum efficiency (ϕ_f = 0.51). Additionally, the synthesis of other azaspiroketal derivatives with increased ring size encountered critical synthetic challenges and afforded products poor yields.

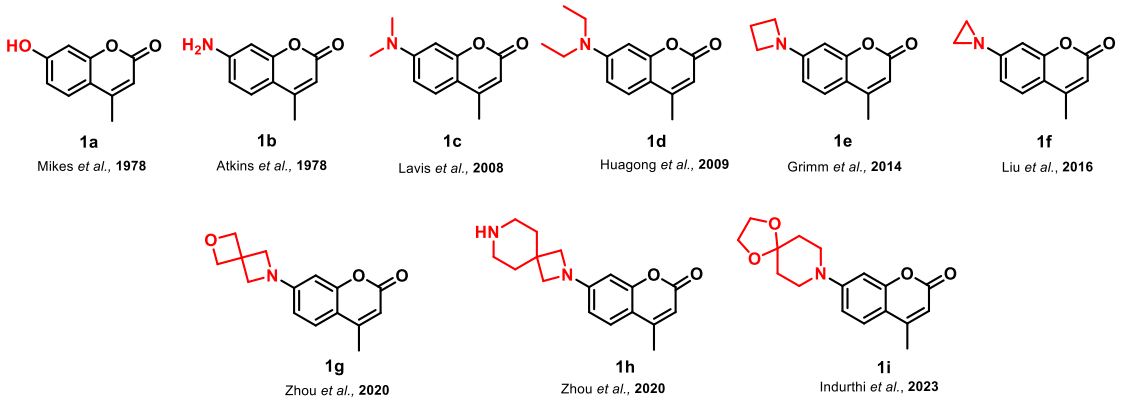

Figure 1.5. Chronological modification of auxochromes on coumarins.

The fluorophoric effects observed in 1,8-naphthalimide (**2a-2g**) (figure 1.7) followed a similar trend when modified with the same set of auxochromes. Substitution of 6-amino and 6-dimethyl amino donating groups of **2a** (ϕ_f<0.1) and **2b** (ϕ_f=0.009)**,** respectively, with a TICT-resistant azetidine ring led to drastic enhancement of quantum efficiency of the resulting compound **2c** (ϕ_f=0.631). A similar observation was made in the case of constrained aziridine ring-substituted derivative **2d** (ϕ_f=0.708). The lack of solubility of 2c was addressed by the incorporation of spiroazetidine rings, as seen in **2e** and **2f** derivatives showcasing enhanced solubility. Nevertheless, subpar quantum yields, expensive substrates and synthetic complications of **2e-2f** led to the discovery of a cost-effective and soluble azaspiroketal-substituted derivative, **2g.**[10]

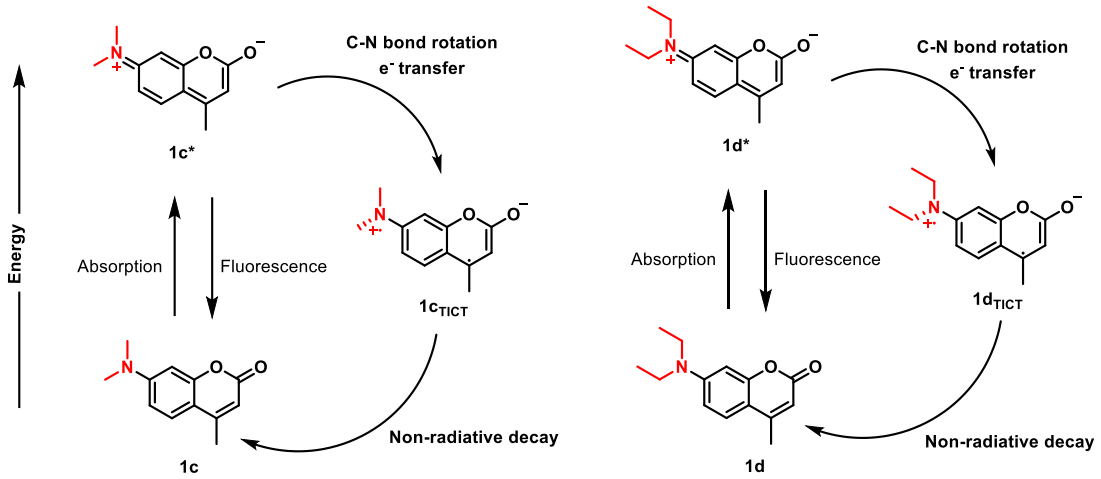

Figure 1.6. Jabłonski diagram showing the process of twisted intramolecular charge transfer (TICT) in compounds 1c and 1d.

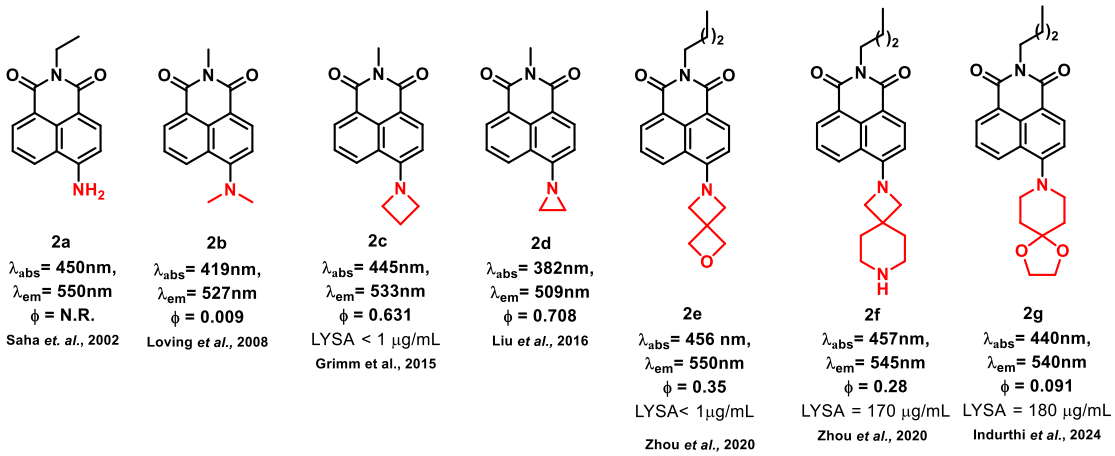

Figure 1.7. Chronological modification of auxochromes on 1,8-naphthalimide.

A consistent trend was also noted in the case of 4-nitrobenzoxadiazole (**3a-3g**) substituted with the identical set of auxochromes as done in previous cases (figure 1.8).

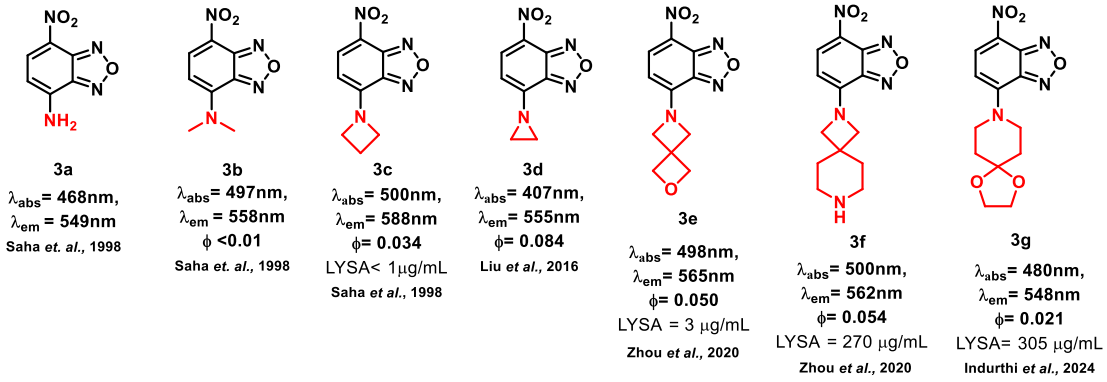

Figure 1.8. Chronological modification of auxochromes on 4-nitrobenzoxadiazole.

Thus, upon review of the paragraph, it becomes evident that even a minor alteration in the auxochrome units can exert a significant impact on the photophysical behaviour of fluorescent dyes.

1.3. Fluorescent Probes and their Role in Bioimaging

Biological systems are highly dynamic, and the processes that take place inside them are random and rapid. The molecular level of understanding of these fast and complicated cellular processes requires specially designed tools for gaining knowledge about them. These tools, known as **fluorescent probes (or detectors)**, include fluorophore-borne small molecules that undergo alterations in their fluorescent emission as a consequence of even a minute modification in their immediate environment, indicating a binding event or a chemical reaction.[11,12] Over the past few decades, fluorescent probes have become widely popular for tracking and monitoring various biological processes. These processes include cellular dynamics, protein expression and localization, enzyme activities, and regulation of signalling cascades. Therefore, it is crucial to identify suitable fluorophores with efficient auxochromes that can be used as probes. The main requirements for a fluorophore to function as a probe include high quantum efficiency, excellent brightness index, good photostability, balanced solubility, and the ability to permeate cells. These requirements have motivated researchers to

create complex fluorescent molecules with exceptional fluorogenic properties, which can be effectively used in the field of bioimaging.

1.3.1. Recent Developments in Fluorescent Probes: Applications and Drawbacks

While conducting a literature survey on various fluorophore-based fluorescent probes, such as coumarins,[12,13] 1,8-naphthalimides[14] and 4-nitrobenzoxadiazoles,[15] we discovered that these probes have numerous applications in detecting and imaging small molecule thiols such as cysteine (Cys), homocysteine (Hcy), and glutathione (GSH). Abnormal levels of these thiols can indicate various physiological disorders, including edema, lethargy, cognitive dysfunction, Alzheimer's disease, hepatic complications, cardiovascular anomalies, and cancer.[13,16,17] These probes can also detect reactive oxygen species (ROS), which play a crucial role in maintaining cellular homeostasis and various cell signaling pathways. [18–24] Hence, a slight disbalance in their levels may also lead to the occurrence of an array of diseases, such as cardiovascular disorders and cancer. Furthermore, these have also been used in the detection of several reducing agents,[25–28] metal toxicants,[29–34] gaseous environmental pollutants [35,36] and also various metabolic enzymes.[37–39] Some of these examples are illustrated in figures 1.9 and 1.10. The probes mentioned above commonly make use of hydroxyl (-OH) or amine ($-NH_2$) groups as their electron-donating auxochromes, as these are the only groups that offer a site for further functionalization with a reactive unit that can interact with the corresponding analytes. Consequently, the resulting fluorescent probes exhibit subpar brightness and poor physicochemical properties and make the process of analyte detection difficult. While the modified amino auxochromes are highly efficient in enhancing photophysical attributes, they all lack the capacity for synthetic modulation and fail to facilitate the attachment of response units required to develop a fluorescent probe. Despite such advancements in auxochrome units, scientists continue to rely on conventional hydroxyl or amino auxochromes for the attachment of response/recognition units.

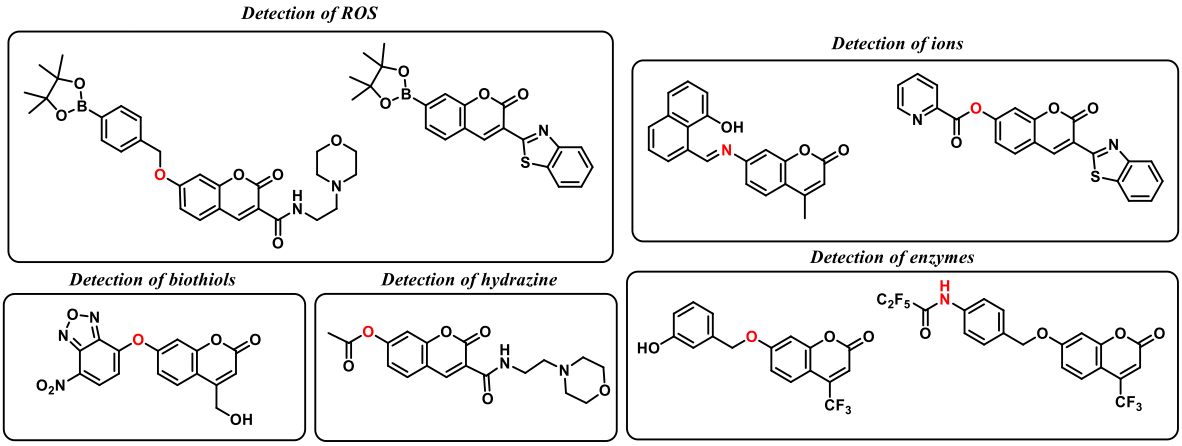

Figure 1.9. Examples of coumarin-based fluorescent probes used for the detection of various analytes.

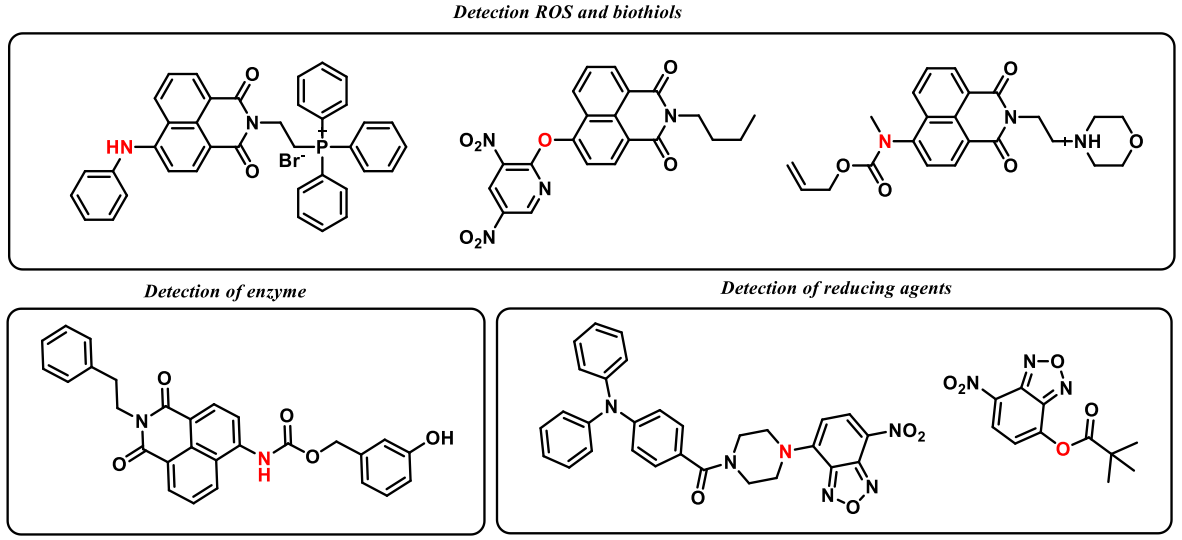

Figure 1.10. Examples of 1,8-naphthalimide and 4-nitrobenzoxadiazole-based fluorescent probes used for the detection of various analytes.

1.4. Conclusion

Fluorescent dyes play a critical role in cell and organelle imaging. All fluorescent dyes are comprised of fluorophores and auxochromes. The auxochrome units have a significant impact on the light-emitting behaviour of these fluorescent dyes. Traditionally, hydroxyl and amine auxochromes have been extensively utilized. However, the past decade has seen the discovery

of azacyclic amines, particularly azetidine, which have demonstrated improved light-emitting properties compared to amines and dialkylamines. It is crucial to highlight that these azacyclic amines lack a point of attachment for a recognition moiety, a vital element in the development of fluorescent probes for biomarker detection. As of now, the attachment of recognition units for various biomarkers still relies on the traditional -OH or -NH_2 groups on the auxochrome unit.

1.5. References

(1) Fluorescence Spectroscopy Principles. In *Principles and Applications of Fluorescence Spectroscopy*; John Wiley & Sons, Ltd, **2007**; pp 88–114. https://doi.org/10.1002/9780470692059.ch7.

(2) Jablonski, A. Über Den Mechanismus Der Photolumineszenz von Farbstoffphosphoren. **1935**, *94*, 38–64.

(3) Süel, G. Chapter Thirteen - Use of Fluorescence Microscopy to Analyze Genetic Circuit Dynamics. In *Methods in Enzymology*; Voigt, C., Ed.; Synthetic Biology, Part A; Academic Press, **2011**; Vol. 497, pp 275–293. https://doi.org/10.1016/B978-0-12-385075-1.00013-5.

(4) *CF® Dyes. What started it all? Part 1. A History of Fluorescence - Biotium*. https://biotium.com/blog/cf-dyes-what-started-it-all-part-1-a-history-of-fluorescence/ (accessed 2024-08-06).

(5) Langhals, H. Fluorescence and Fluorescent Dyes. *Phys. Sci. Rev.* **2020**, *5* (8). https://doi.org/10.1515/psr-2019-0100.

(6) Grimm, J. B.; Lavis, L. D. Caveat Fluorophore: An Insiders' Guide to Small-Molecule Fluorescent Labels. *Nat. Methods* **2022**, *19* (2), 149–158. https://doi.org/10.1038/s41592-021-01338-6.

(7) Gandioso, A.; Bresolí-Obach, R.; Nin-Hill, A.; Bosch, M.; Palau, M.; Galindo, A.; Contreras, S.; Rovira, A.; Rovira, C.; Nonell, S.; Marchán, V. Redesigning the Coumarin Scaffold into Small Bright Fluorophores with Far-Red to Near-Infrared Emission and Large Stokes Shifts Useful for Cell Imaging. *J. Org. Chem.* **2018**, *83* (3), 1185–1195. https://doi.org/10.1021/acs.joc.7b02660.

(8) Zhou, J.; Lin, X.; Ji, X.; Xu, S.; Liu, C.; Dong, X.; Zhao, W. Azetidine-Containing Heterospirocycles Enhance the Performance of Fluorophores. *Org. Lett.* **2020**, *22* (11), 4413–4417. https://doi.org/10.1021/acs.orglett.0c01414.

(9) Indurthi, H. K.; Goswami, P.; Das, S.; Saha, P.; Koch, B.; Sharma, D. K. 7-Azaspiroketal as a Unique and Effective Auxochrome Moiety: Demonstration in a Fluorescent Coumarin Dye and Application in Cell Imaging. *New J. Chem.* **2023**, *47* (47), 21608–21611. https://doi.org/10.1039/D3NJ04934E.

(10) Indurthi, H. K.; Goswami, P.; Das, S.; Saha, P.; Koch, B.; Sharma, D. K. Azaspiroketal as Novel Auxochrome for Enhancing the Fluorescence Brightness and Solubility of Naphthalimide and Nitrobenzoxadiazole Dyes. *Chem. Sel.* **2024**, *9* (26), e202401523. https://doi.org/10.1002/slct.202401523.

(11) Das, S.; Indurthi, H. K.; Asati, P.; Saha, P.; Sharma, D. K. Benzothiazole Based Fluorescent Probes for the Detection of Biomolecules, Physiological Conditions, and Ions Responsible for Diseases. *Dyes Pigm.* **2022**, *199*, 110074. https://doi.org/10.1016/j.dyepig.2021.110074.

(12) Das, S.; Indurthi, H. K.; Saha, P.; Sharma, D. K. Coumarin-Based Fluorescent Probes for the Detection of Ions, Biomolecules and Biochemical Species Responsible for Diseases. *Dyes Pigm.* **2024**, *228*, 112257. https://doi.org/10.1016/j.dyepig.2024.112257.

(13) Cao, D.; Liu, Z.; Verwilst, P.; Koo, S.; Jangjili, P.; Kim, J. S.; Lin, W. Coumarin-Based Small-Molecule Fluorescent Chemosensors. *Chem. Rev.* **2019**, *119* (18), 10403–10519. https://doi.org/10.1021/acs.chemrev.9b00145.

(14) Han, C.; Sun, S.-B.; Ji, X.; Wang, J.-Y. Recent Advances in 1,8-Naphthalimide-Based Responsive Small-Molecule Fluorescent Probes with a Modified C4 Position for the Detection of Biomolecules. *Trends Anal. Chem.* **2023**, *167*, 117242. https://doi.org/10.1016/j.trac.2023.117242.

(15) Jiang, C.; Huang, H.; Kang, X.; Yang, L.; Xi, Z.; Sun, H.; Pluth, M. D.; Yi, L. NBD-Based Synthetic Probes for Sensing Small Molecules and Proteins: Design, Sensing Mechanisms and Biological Applications. *Chem. Soc. Rev.* **2021**, *50* (13), 7436–7495. https://doi.org/10.1039/D0CS01096K.

(16) Yin, B.; Sha Li, S.; Hua Lu, S.; Ying Mi, J.; Lin Zhai, H. Simultaneous Quantification of Multiple Endogenous Biothiols in Cancer Cells Based on a Multi-Signal Fluorescent Probe. *Analyst* **2019**, *144* (15), 4575–4581. https://doi.org/10.1039/C9AN00691E.

(17) Słowiński, D.; Świerczyńska, M.; Romański, J.; Podsiadły, R. HPLC Study of Product Formed in the Reaction of NBD-Derived Fluorescent Probe with Hydrogen Sulfide, Cysteine, N-Acetylcysteine, and Glutathione. *Molecules* **2022**, *27* (23), 8305. https://doi.org/10.3390/molecules27238305.

(18) Zuo, Y.; Jiao, Y.; Ma, C.; Duan, C. A Novel Fluorescent Probe for Hydrogen Peroxide and Its Application in Bio-Imaging. *Molecules* **2021**, *26* (11), 3352. https://doi.org/10.3390/molecules26113352.

(19) Yao, W.; Cao, Y.; She, M.; Yan, Y.; Li, J.; Leng, X.; Liu, P.; Zhang, S.; Li, J. Imaging and Monitoring the Hydrogen Peroxide Level in Heart Failure by a Fluorescent Probe with a Large Stokes Shift. *ACS Sens.* **2021**, *6* (1), 54–62. https://doi.org/10.1021/acssensors.0c01707.

(20) Wang, Q.-M.; Jin, L.; Shen, Z.-Y.; Xu, J.-H.; Sheng, L.-Q.; Bai, H. Mitochondria-Targeting Turn-on Fluorescent Probe for HClO Detection and Imaging in Living Cells. *Spectrochim. Acta Mol. Biomol. Spectrosc.* **2020**, *228*, 117825. https://doi.org/10.1016/j.saa.2019.117825.

(21) He, X.; Chen, H.; Xu, C.; Fan, J.; Xu, W.; Li, Y.; Deng, H.; Shen, J. Ratiometric and Colorimetric Fluorescent Probe for Hypochlorite Monitor and Application for Bioimaging in Living Cells, Bacteria and Zebrafish. *J. Hazard. Mater.* **2020**, *388*, 122029. https://doi.org/10.1016/j.jhazmat.2020.122029.

(22) An, Z.; Zhao, Z.; Zhao, L.; Yue, Q.; Li, K.; Zhao, B.; Miao, J.; Su, L. The Novel HOCl Fluorescent Probe CAN Induced A549 Apoptosis by Inhibiting Chlorination Activity of MPO. *Bioorg. Med. Chem. Lett.* **2020**, *30* (18), 127394. https://doi.org/10.1016/j.bmcl.2020.127394.

(23) Wang, Y.; Ding, F.; Sun, X.; Chen, S.; Huang, H.; Chen, H. A Reaction-Based Colorimetric and Ratiometric Chemosensor for Imaging Identification of HClO in Live Cells, Mung Bean Sprouts, and Paper Strips. *Talanta* **2021**, *234*, 122655. https://doi.org/10.1016/j.talanta.2021.122655.

(24) Xia, L.; Tong, Y.; Li, L.; Cui, M.; Gu, Y.; Wang, P. A Selective Fluorescent Turn-on Probe for Imaging Peroxynitrite in Living Cells and Drug-Damaged Liver Tissues. *Talanta* **2019**, *204*, 431–437. https://doi.org/10.1016/j.talanta.2019.06.032.

(25) Tong, X.; Hao, L.; Song, X.; Wu, S.; Zhang, N.; Li, Z.; Chen, S.; Hou, P. A Fast-Responsive Fluorescent Probe Based on a Styrylcoumarin Dye for Visualizing Hydrogen

Sulfide in Living MCF-7 Cells and Zebrafish. *RSC Adv.* **2022**, *12* (28), 17846–17852. https://doi.org/10.1039/D2RA00997H.

(26) Shi, G.-J.; Wang, Y.-D.; Yu, Z.-X.; Zhang, Q.; Chen, S.; Xu, L.-Z.; Wang, K.-P.; Hu, Z.-Q. The Coumarin-Pyrazole Dye for Detection of Hydrogen Sulfide in Cells. *Spectrochim. Acta Mol. Biomol. Spectrosc.* **2023**, *285*, 121898. https://doi.org/10.1016/j.saa.2022.121898.

(27) Zhang, X.; Yang, Y.; Zhang, L.; Liu, S.; Song, Z.; Zhang, L.; You, J.; Chen, L. Development of Fluorescent Probes with Specific Recognition Moiety for Hydrogen Polysulfide. *Talanta* **2024**, *268*, 125293. https://doi.org/10.1016/j.talanta.2023.125293.

(28) He, X.; Xu, W.; Ding, F.; Xu, C.; Li, Y.; Chen, H.; Shen, J. Reaction-Based Ratiometric and Colorimetric Chemosensor for Bioimaging of Biosulfite in Live Cells, Zebrafish, and Food Samples. *J. Agric. Food Chem.* **2020**, *68* (42), 11774–11781. https://doi.org/10.1021/acs.jafc.0c03983.

(29) Hien, N. K.; Bay, M. V.; Bao, N. C.; Vo, Q. V.; Cuong, N. D.; Thien, T. V.; Nhung, N. T. A.; Van, D. U.; Nam, P. C.; Quang, D. T. Coumarin-Based Dual Chemosensor for Colorimetric and Fluorescent Detection of Cu^{2+} in Water Media. *ACS Omega* **2020**, *5* (33), 21241–21249. https://doi.org/10.1021/acsomega.0c03097.

(30) Chen, H.; Yang, P.; Li, Y.; Zhang, L.; Ding, F.; He, X.; Shen, J. Insight into Triphenylamine and Coumarin Serving as Copper (II) Sensors with “OFF” Strategy and for Bio-Imaging in Living Cells. *Spectrochim. Acta Mol. Biomol. Spectrosc.* **2020**, *224*, 117384. https://doi.org/10.1016/j.saa.2019.117384.

(31) Ahmed, N.; Zareen, W.; Zhang, D.; Yang, X.; Ye, Y. Coumarin-Based Reversible Fluorescent Probe for Selective Detection of Cu^{2+} in Living Cells. *J. Fluoresc.* **2020**, *30* (5), 1171–1179. https://doi.org/10.1007/s10895-020-02585-0.

(32) Ngororabanga, J. M. V.; Tshentu, Z. R.; Mama, N. A New Highly Selective Colorimetric and Fluorometric Coumarin-Based Chemosensor for Hg^{2+}. *J. Fluoresc.* **2020**, *30* (5), 985–997. https://doi.org/10.1007/s10895-020-02542-x.

(33) Khan, M. A.; Meena, S.; Alam, Md. A.; Ghosh, S. A Solvent Sensitive Coumarin Derivative Coupled with Gold Nanoparticles as Selective Fluorescent Sensor for Pb2+ Ions in Real Samples. *Spectrochim. Acta Mol. Biomol. Spectrosc.* **2020**, *243*, 118810. https://doi.org/10.1016/j.saa.2020.118810.

(34) Romano, G. M.; Zizi, V.; Salvatore, G.; Bani, R.; Mangoni, M.; Nistri, S.; Anichini, G.; Simonini Steiner, Y. T.; Bani, D.; Bianchi, A.; Bencini, A.; Savastano, M. Evaluation of Coumarin-Tagged Deferoxamine as a Zr(IV)-Based PET/Fluorescence Dual Imaging Probe. *J. Inorg. Biochem.* **2023**, *245*, 112259. https://doi.org/10.1016/j.jinorgbio.2023.112259.

(35) Jiang, W.-L.; Wang, W.-X.; Mao, G.-J.; Yan, L.; Du, Y.; Li, Y.; Li, C.-Y. Construction of NIR and Ratiometric Fluorescent Probe for Monitoring Carbon Monoxide under Oxidative Stress in Zebrafish. *Anal. Chem.* **2021**, *93* (4), 2510–2518. https://doi.org/10.1021/acs.analchem.0c04537.

(36) Zhang, S.; Mu, X.; Zhu, J.; Yan, L. A Metal-Free Coumarin-Based Fluorescent Probe for the Turn-on Monitoring of Carbon Monoxide in an Aqueous Solution and Living Cells. *Analyst* **2021**, *146* (4), 1289–1294. https://doi.org/10.1039/D0AN02107E.

(37) Dockalova, V.; Sanchez-Carnerero, E. M.; Dunajova, Z.; Palao, E.; Slanska, M.; Buryska, T.; Damborsky, J.; Klán, P.; Prokop, Z. Fluorescent Substrates for Haloalkane Dehalogenases: Novel Probes for Mechanistic Studies and Protein Labeling. *Comput. Struc. Biotechnol. J.* **2020**, *18*, 922–932. https://doi.org/10.1016/j.csbj.2020.03.029.

(38) Sun, Q.; Guo, Y.; Li, X.; Luo, X.; Qiu, Y.; Liu, G. A Tyrosinase Fluorescent Probe with Large Stokes Shift and High Fluorescence Enhancement for Effective Identification of Liver Cancer Cells. *Spectrochim. Acta Mol. Biomol. Spectrosc.* **2023**, *285*, 121831. https://doi.org/10.1016/j.saa.2022.121831.

(39) Sun, Q.; Li, X.; Guo, Y.; Qiu, Y.; Luo, X.; Liu, G.; Han, Y. Coumarin-Based Turn-on Fluorescence Probe with a Large Stokes Shift for Detection of Endogenous Neutrophil Elastase in Live Cells and Zebrafish. *Spectrochim. Acta Mol. Biomol. Spectrosc.* **2022**, *281*, 121563. https://doi.org/10.1016/j.saa.2022.121563.