SYNTHESIS AND EVALUATION OF FLUORESCENT TOOLS FOR STUDIES OF CANCER BIOLOGY

***Murthaeti Yaswanth(Corresponding Author), **Brij Kishore Tiwari, ***Dr.G.S.Gayathri, ****Dr. Kunal V.Bhambar, *****Syed. Sadaq Valli, ******Dr. J. Saravanan**

***Assistant Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, PES University, Hanumantha Nagar, Bangalore, Karnataka, India, Email: murthaetiyaswanth@pes.edu**

****Assistant Professor, Department of Applied Sciences, G L Bajaj Institute of Technology and Management, Greater Noida, U.P., India, Email: brijktiwari01@gmail.com**

***** Associate Professor, Department of Chemistry, S. A. Engineering College, Chennai, Tamil Nadu, India, Email: gayathrigs@saec.ac.in**

******Assistant Professor, Department of Pharmacy, Savitribai Phule Pune University, Pune, Maharashtra, India, Email: kunalbhambar@gmail.com**

*******Research Associate, Department of Pharmaceutical Analysis, Aurigene Oncology, Bangalore, Karnataka, India, Email: sadaqvallisyed@gmail.com**

********Dean of Pharmaceutical Sciences & Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, PES University, Hanumantha Nagar, Bangalore, Karnataka, India, Email: saravananj@pes.edu**

Abstract

In this paper, the researcher looked at the synthesis mechanisms and the working principle of different fluorophores within the human body in a brief manner. The early detection of cancer cells was necessary for the detection and treatment of cancer patients. Previously, surgical procedures, as well as radiological imaging were widely used for the detection of cancer cells. But, in recent years fluorescence imaging for the detection of cancer cells become widely popular. The process works on the principle that, the fluorophores which are tagged with proteins or antibodies, were bind to the enzymes or proteins involved in the completion of a cell cycle. Various fluorophores like “fluorescence green”, “fluorescence orange”, and “fluorescence red”, as well as methylene blue, indocyanine green, and fluorescein are widely used. Once the fluorophores bind with the target proteins, they started to emit excited fluorescent light, these light waves were detected by the fluorescent detector present at the end of the machine. However, the synthesis of fluorophore molecules involves very complex mechanisms. Any deviation of the level of those from a normal standard can help physicians to identify the cancer cells.

Keywords- FS, FP, RD, MTB, FC, IG, ICG

1.0 Introduction

Cancer is a serious and practically non-curable disease. The main reason behind the start and dominance of cancer in human cells, is because of faults in the cell cycle. Typically, the control mechanisms established in the body to stop the unregulated growth of cells gets hampered. As a result, the cells start to grow in an indefinite manner and replace the healthy cells. There are various tools and mechanisms available today to erect such cells in the early stages of cancer. One such tool used for the detection of such cells are fluorescent objects. These are biochemical molecules, which when bind with the cancer cells through a tagging mechanism, detected under the fluorescence spectroscopy mechanisms.

2.0 Discussion

2.1 Synthesis of fluorescent tools

It is well-established that defects in the cell cycle lead to the development of cancer. In recent years, various fluorescence chemosensors were deployed to monitor the activities of various enzymes and constituents of biomolecules. These are very small molecules, which are usually tagged with the biochemical molecule, and bind to the enzymes. Generally, these molecules are also used for targeted cancer cell therapies and gene therapies of cancer cells. In general theory, any antibody or biochemical small molecules targeting cancer cells were labeled with fluorescent agents (Obara*et al.* 2021). As a result, bioluminescence imaging and fluorescence imaging were done to identify the cancer cells, the mechanisms of enzymes and proteins involved in the control mechanism of the cell cycle among others. The components of fluorescence imaging include a biologically safe fluorescence protein and optical detectors which are highly sensitive to these proteins. The fluorophores include "fluorescence green", "fluorescence orange", and "fluorescence red" among others (Chilka*et al.* 2019). The most commonly used fluorophore operated in the infrared region and used for the clinical use are methylene blue, indocyanine green, and fluorescein. Their working behavior *in vivo* depends mainly on the amount of dose injected, metabolic activities inside the body, affinity targeting, and biodistribution.

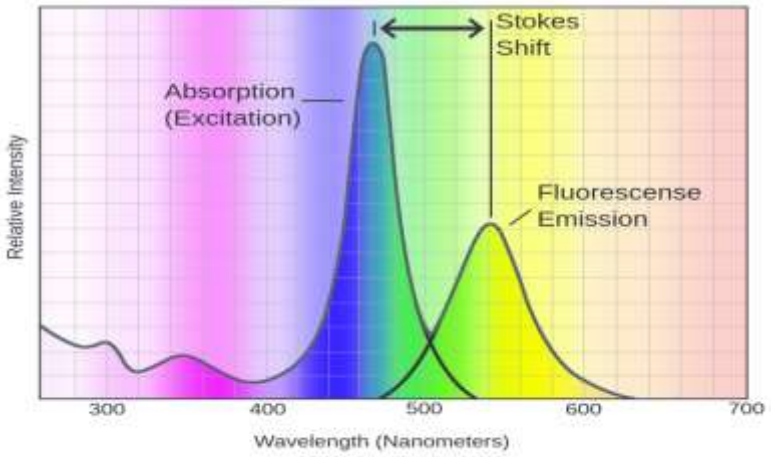


Figure 1: Graph showing excitation of fluorophores at greater wavelength

(Source: theory.labster.com)

The synthesis of the fluorophore "CH1055" from "benzo-bisthiadiazole (BBTD)" is a common process. The majority of them had a BBTD core or were symmetrical fluorophores with a "D–A–D structure". The LUMO showed a strong contribution as the electron-accepting material,
And act as the fluorophore. ***[Refer to appendix 1]***

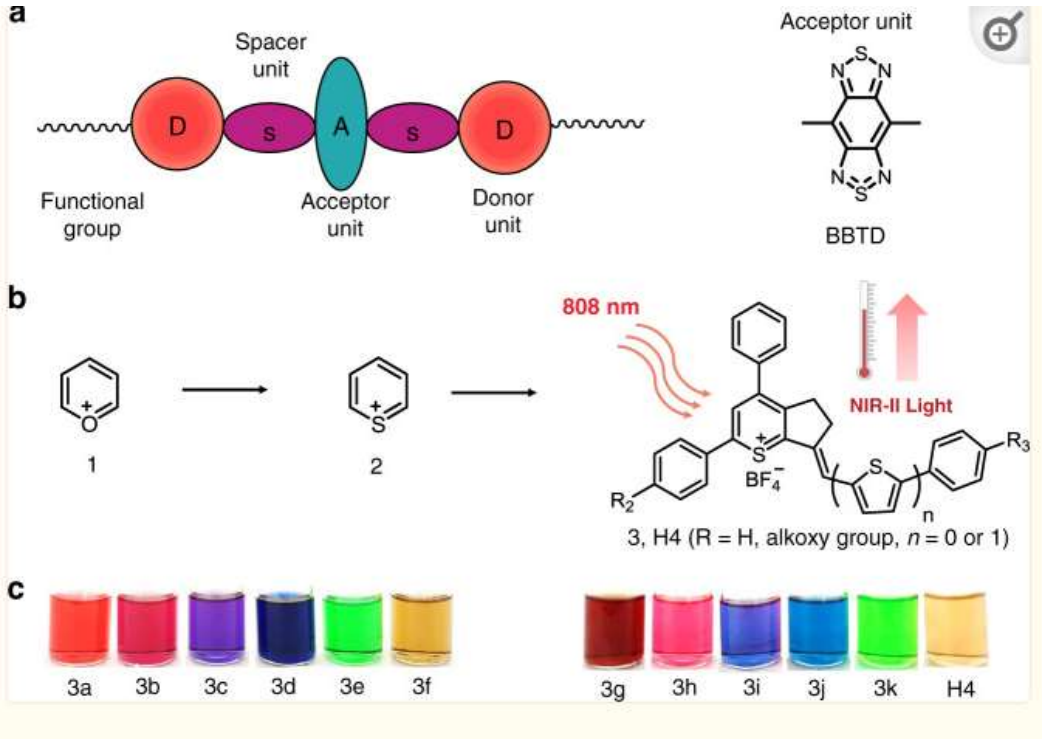


Figure 2: Mechanism of fluorophore synthesis

(Source: www.ncbi.nlm.nih.gov)

Functional groups in the NIR-II molecules at the core of the BBTD molecule absorb the light emitted by the fluorophore molecules and images of the bright field nature of a series of new "thiopyrylium-based probes" were observed (Zhang *et al.* 2019). Among all the dyes of fluorescent nature, "FUCL (fluorescence upconversion luminescence materials)" are very important. These molecules convert low energy to high energy emission by the excitation process.

The dye followed the D-A-D structure and in the first stage (a) reagents like benzaldehyde, acetophenone, aqueous KOH, and EtOHwre used. Also, compounds like bromoprop-1-yne, acetone, and K_2CO_3 were used. In the second step (b) compounds of pyrrolidine, cyclopentanone, and benzene were used. In the third step c, "thioacetic acid", "boron trifluoride ether", and "ether" were used. In the fourth step (d), acetic anhydrides, and aldehydes were used (Yin *et al.* 2019). The key intermediate "thiopyryliumtetrafluoroborate" was obtained, then under microwave irradiation conditions subsequent condensation of aldehydes resulted in the formation of the target fluorophore. ***[Refer to appendix 2]***

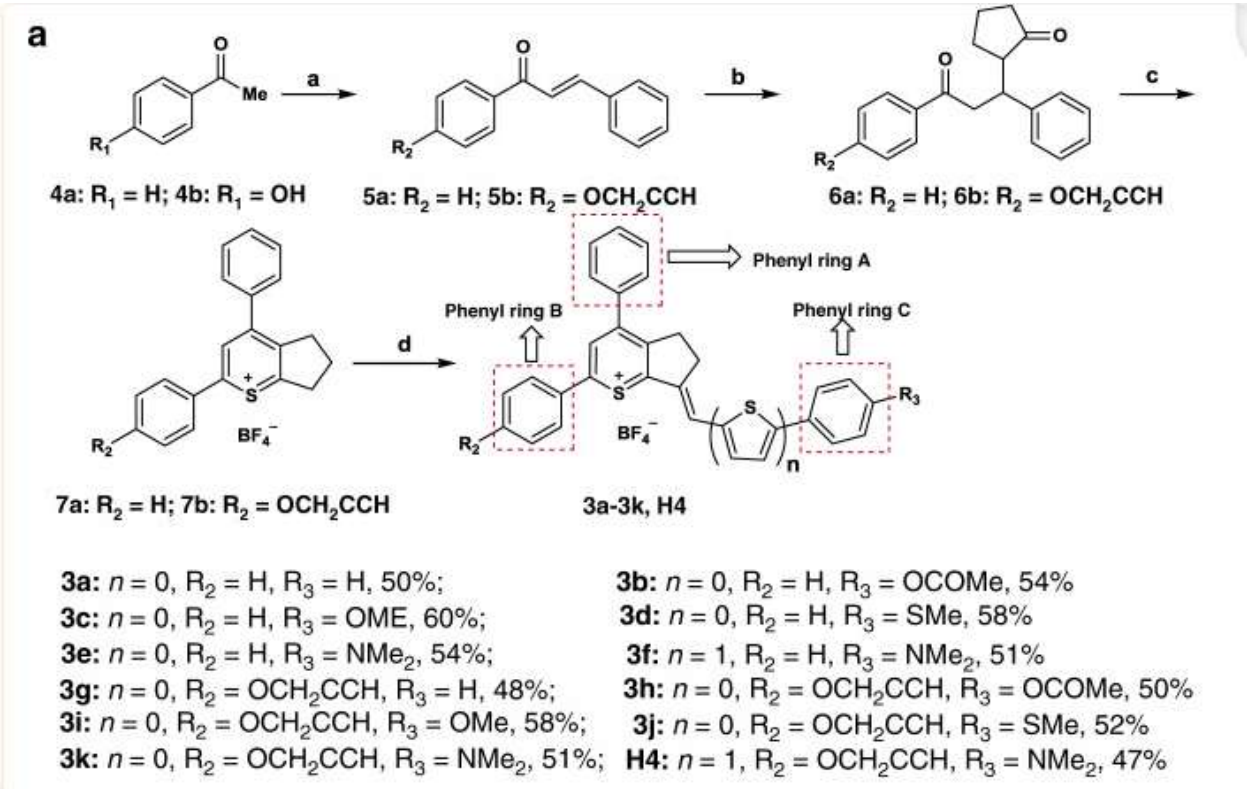


Figure 3: Mechanism of fluorophore synthesis
(Source: www.ncbi.nlm.nih.gov)

2.2 Evaluation of fluorescent tools

There are various methods available to detect the cancer cells in the early stages of cancer. These methods include surgical procedures, as well as radiological imaging tools. But, fluorescent tagged materials are increasingly used for the identification of cancer cells, in the patients. Theoretically, "bioluminescent" or fluorescent agents were labeled on the antibodies or small protein molecules that target the cancer cells. The imaging technique is particularly useful for those patients who have an early secondary disease, which limits the option of surgical procedures. The flurophore molecules are tagged with protein or other biochemical agents, which bind to the enzymes or proteins deeply involved in the cell cycle procedure (Luo *et al.* 2019). Once, they had binded, with the protein molecules, they started to emit fluorescence lights, which were then detected by an optical wave detector. The fluorophores include "fluorescence green", "fluorescence orange", and "fluorescence red" among others. The most commonly used fluorophore operated in the infrared region and used for the clinical use are methylene blue, indocyanine green, and fluorescein. Their working behavior *in vivo* depends mainly on the amount of dose injected, metabolic activities inside the body, affinity targeting, and biodistribution. The fluorophore "Fluorescein sodium" emits fluorescent light at 510-520 nm wavelength, "Methylene blue" emits fluorescent light at 690 nm, and "Indocyanine blue" emits

fluorescent light at 800-850 nm (Halicek*et al.* 2019). The device which completes the light emission and subsequent detection is composed of a light source, usually with a variety of wavelengths. To eliminate excited or ambient lights for use with one or more fluorophores filters are inserted into the device. The detector of the appropriate spectral range and sensitivity, along with a good visual for real-time fluorescent readout superimposed on a reference image, are also essential.

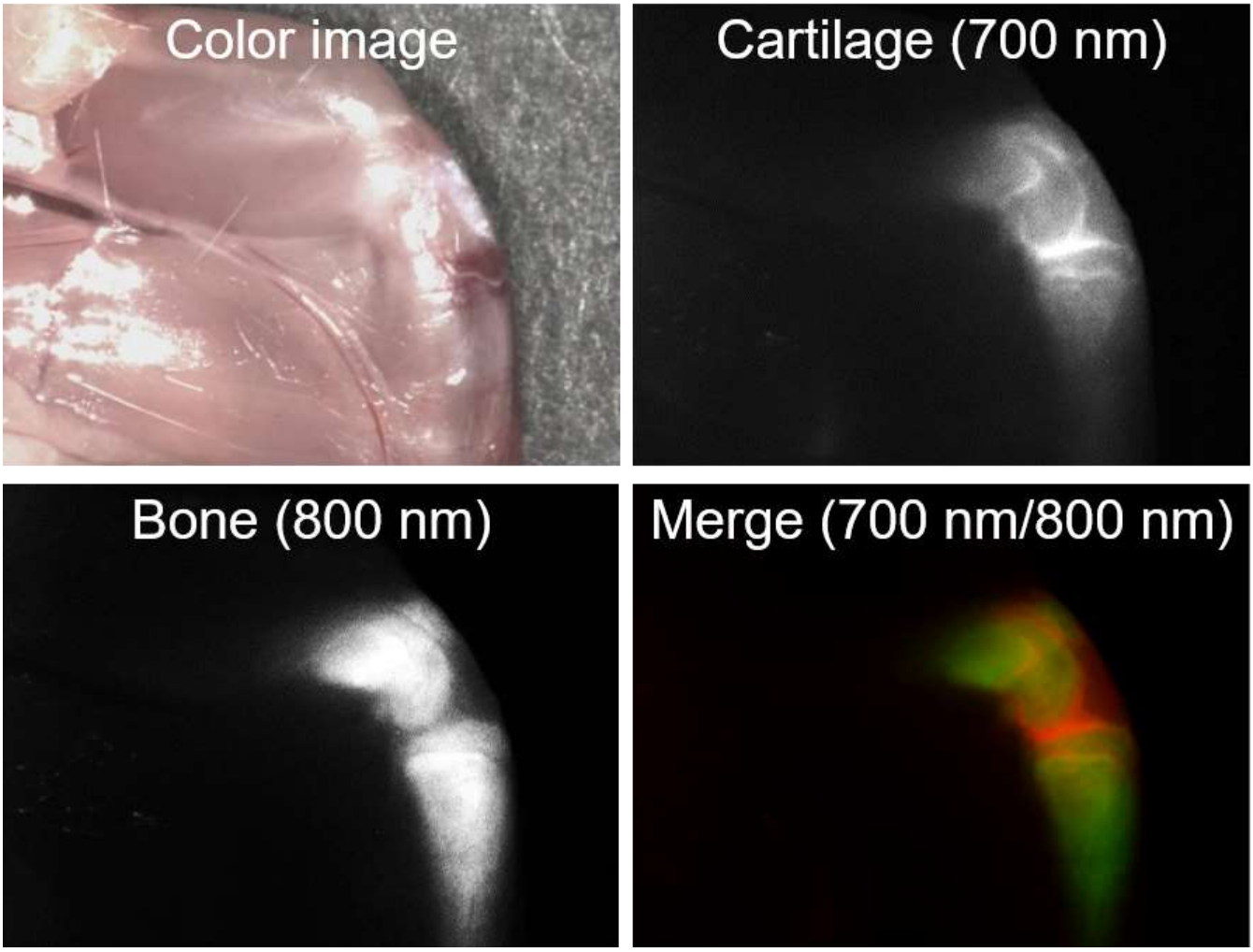


Figure 4: Small molecule fluorophores

(Source: projects.iq.harvard.edu)

Sometimes, different fluorescent dyes are used to highlight the body sections which developed tumors, the early stage of developing cancer. The principle is based on the fact that the tumor should be as such in which blood flows at a very high level. Sodium FL has been used for this purpose (Baeten*et al.* 2022). The detection of tumors using the ICG fluorescence follow a different principle. After injection of the ICG material, the liver as a result of dye metabolism becomes fluorescence green. But, the tumors stayed non-fluorescence nearly for 1 hour. During

this time, the dye was also exerted by the liver into the biliary system (Campbell *et al.* 2019). This leads to the detection of fluorescence differential in the liver and tumors was identified.

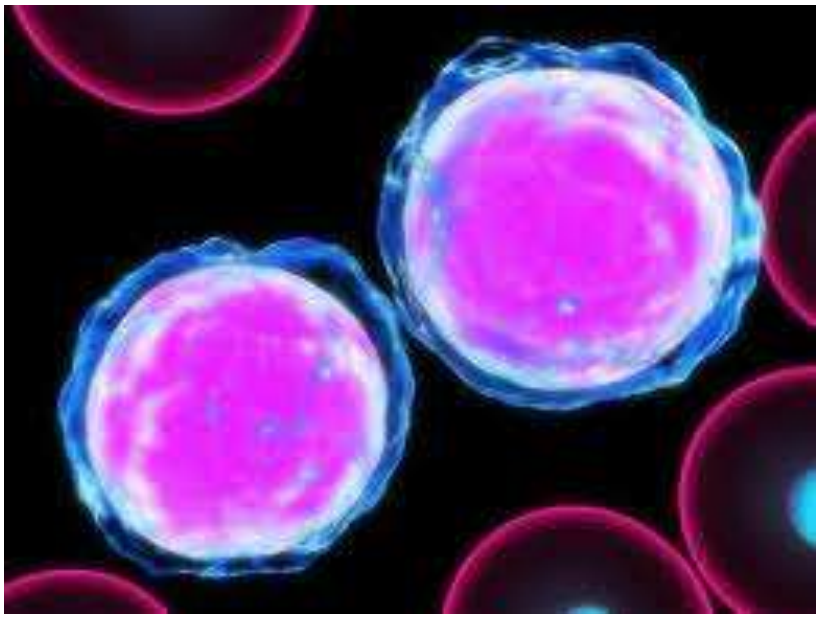


Figure 5: Cancer cells after flurophores staining

(Source: www.genengnews.com)

In the image below, the first (A) image shows the presence of a tumor in the liver, prior to any fluorescence activity. The second (B) image shows the delineation of liver cells by the ICG fluorescence. The third image C showed, the tumor cells in a liver from an edge cut. And, the fourth image (D) shows, the negative margin of fluorescence display of the liver cells.

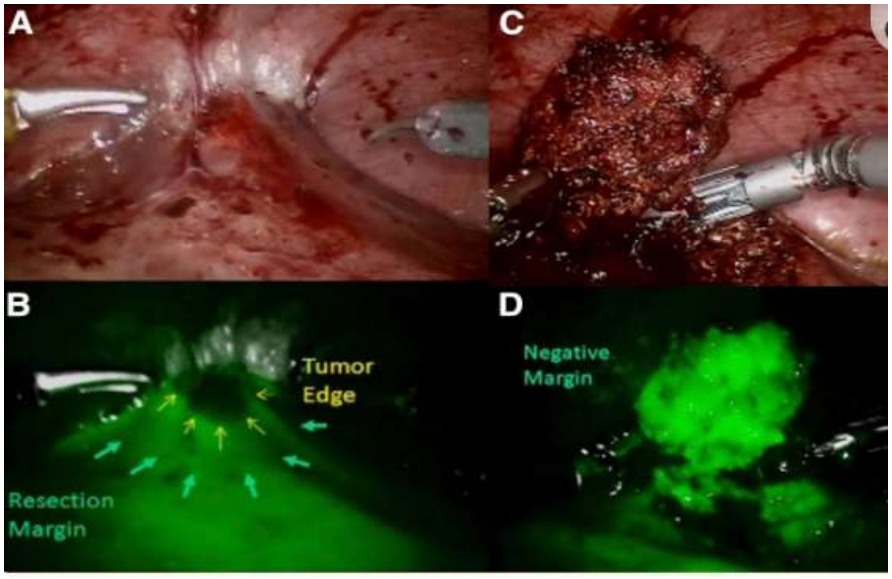


Figure 3: Display of liver cells before and after fluorescence staining

(Source: www.ncbi.nlm.nih.gov)

3.0 Conclusion

Cancer is a very serious, and almost non-curable disease in humans. Thus, an early detection of these cells are essential for the patient survival. Various methods, including the surgical procedures, the detection of cancer cells using radiological methods were long been in use. But, nowadays new techniques of fluorescence imaging of those cells are widely popular and used. This process does not risk patients being exposed to radiation-related harm, as well as surgical operations. fluorophores include "fluorescence green", "fluorescence orange", and "fluorescence red", as well as methylene blue, indocyanine green, and fluorescein are widely used. The process works on the principle that, the fluorophores which are tagged with proteins or antibodies, were bind to the enzymes or proteins involved in the completion of a cell cycle. Therefore, any deviation of the level of those from a normal standard can help physicians to identify the cancer cells.

Reference List

Journals

Baeten, I.G., Hoogendam, J.P., Jeremiasse, B., Braat, A.J., Veldhuis, W.B., Jonges, G.N., Jürgenliemk- Schulz, I.M., van Gils, C.H., Zweemer, R.P. and Gerestein, C.G., 2022. Indocyanine green versus technetium- 99m with blue dye for sentinel lymph node detection in early- stage cervical cancer: A systematic review and meta- analysis. *Cancer Reports*, *5*(1), p.e1401.

Campbell, E., Hasan, M.T., Gonzalez Rodriguez, R., Akkaraju, G.R. and Naumov, A.V., 2019. Doped graphene quantum dots for intracellular multicolor imaging and cancer detection. *ACS Biomaterials Science & Engineering*, *5*(9), pp.4671-4682.

Chilka, P., Desai, N. and Datta, B., 2019. Small molecule fluorescent probes for G-quadruplex visualization as potential cancer theranostic agents. *Molecules*, *24*(4), p.752.

Halicek, M., Dormer, J.D., Little, J.V., Chen, A.Y., Myers, L., Sumer, B.D. and Fei, B., 2019. Hyperspectral imaging of head and neck squamous cell carcinoma for cancer margin detection in surgical specimens from 102 patients using deep learning. *Cancers*, *11*(9), p.1367.

Luo, X., Wang, R., Lv, C., Chen, G., You, J. and Yu, F., 2019. Detection of selenocysteine with a ratiometric near-infrared fluorescent probe in cells and in mice thyroid diseases model. *Analytical chemistry*, *92*(1), pp.1589-1597.

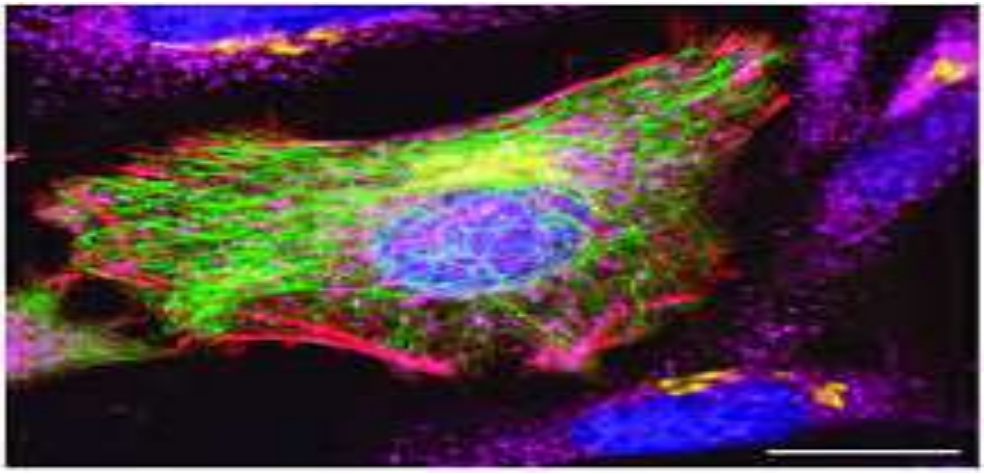
Obara, R., Kamiya, M., Tanaka, Y., Abe, A., Kojima, R., Kawaguchi, T., Sugawara, M., Takahashi, A., Noda, T. and Urano, Y., 2021. γ- Glutamyltranspeptidase (GGT)- activatable fluorescence probe for durable tumor imaging. *AngewandteChemie International Edition*, *60*(4), pp.2125-2129.

Yin, J., Peng, M. and Lin, W., 2019. Visualization of mitochondrial viscosity in inflammation, fatty liver, and cancer living mice by a robust fluorescent probe. *Analytical chemistry*, *91*(13), pp.8415-8421.

Zhang, X., He, N., Huang, Y., Yu, F., Li, B., Lv, C. and Chen, L., 2019. Mitochondria-targeting near-infrared ratiometric fluorescent probe for selective imaging of cysteine in orthotopic lung cancer mice. *Sensors and Actuators B: Chemical*, *282*, pp.69-77.

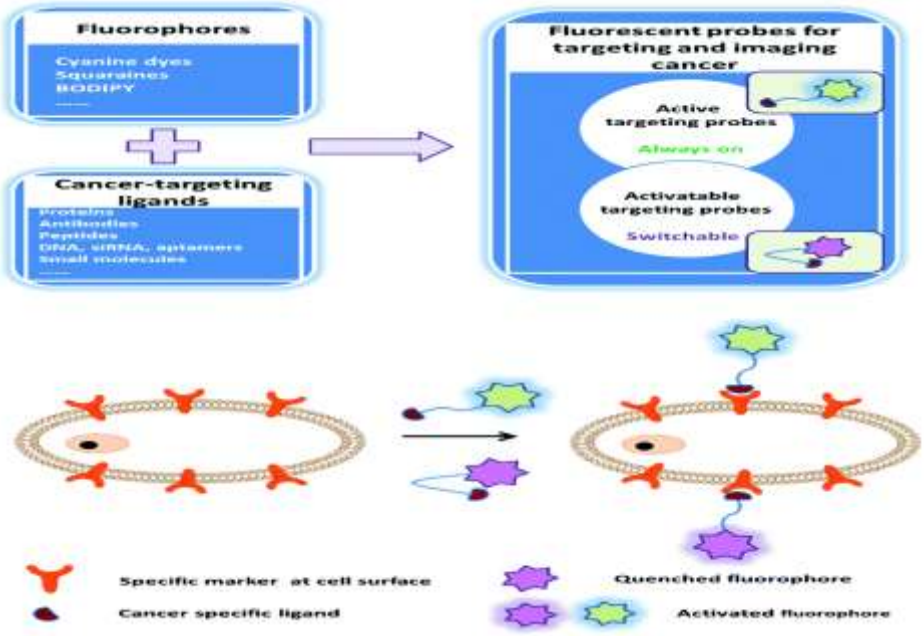
Appendices

Appendix 1: Cancer cells in liver after attachment with the tagged fluorophore



(Source: https://jnanobiotechnology.biomedcentral.com)

Appendix 2: Mechanism of fluorophores targeting cancer cells



(Source: https://pubs.rsc.org)