Aggregation Induced Emission Bio-probes For Medical Research Application

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Declaration

“I certify that this work does no incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text."

Margi Patel
Date: 22/05/2017

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Abstract

Non-invasive and long-term method for cell tracking gives critical information on the cell-based assays like to understand structure, development, distribution and behaviour of cells. Fluorescent probes used in cell tracking to have cell images. These fluorescent probes are powerful tool in the field of cell based research. In this research study we show that, Water-soluble Aggregation Induced Emission (AIE) fluorogens are used to stain the cells because of its high emission efficiency. Even AIE fluorogens have great cell permeability. In this study BSPOTPE AIEgen is used to stain the human embryonic kidney cells due to its great properties like excellent photo-stability and retention time in cell intracellular compartment comparatively with other commercial dyes. The efficient long time cytoplasm retention time of BSPOTPE gives good choice to its suitability in live cell cytoplasm tracker. The AIE dyes performance opens a new era in fluorescence as bio-probes for monitoring cells and its biological process.

Chapter 1: Introduction

Any absorbed light or electromagnetic radiation emitted by substance as visible light or X-rays is called fluorescence and/or phosphorescence. Phosphorescence followed delayed emission by electromagnetic radiation. If the source of radiation stops or remove, the emission of light stops in fluorescence but in phosphorescence the emission of light remains for some time even after removal of radiation source (Dreier, K. 2017). The process in which intensity of fluorescence substance decreases is called quenching (Mei et al.,2014). There are two different types of dyes that are used as cell trackers to find out the activity of cells, their treatments responses and medicine effects on the cell. For cell tracking, there are Aggregation-caused Quenching (ACQ) and Aggregation-induced Emission (AIE) dyes (Mei et al.,2014). In ACQ process, the substances are highly emissive in solution state but they become low emission or sometimes it became non-emissive when aggregate. But it can't be used in solution phase and condensed phase. There is limited application of ACQ mechanism in cell trackers because of its conventional fluorophores (Mei et al.,2014). AIE - aggregation induced emission is a group of fluorescence material are weakly emissive in solution but highly emissive by aggregate formation (Mei et al.,2014).

1.1: Bio-sensors and Bio-probes

Bio-sensors are also called as biological sensors, which are used to know the parameters of the bio-logical elements. This is made up with transducer and liquid fluid like enzyme, nucleic acid or anti-body etc. that converts the biological signals to electrical signals through transducer (News medical life science, 2014). in form of electrical signals or vibrations or images. In every bio-probes or bio-sensors there is one biological element that acts as a sensor and one electronic component that used to detect the electrical signal in different forms (News medical life science, 2014).

. There are several types of biosensors based on the biological element and transducer they used. Enzyme, anti-bodies, biological tissues or cells are different biological element in the bio-sensors. There are several types of transducer like electrochemical, optical, acoustic or calorimetric. Different biosensors used for different purpose. Bio- sensors are used in different areas like in health-care monitoring, detecting some disease, agriculture application and pollution control in the environment (News medical life science , 2014).

As bio-medical research focus is to develop the device that helps to monitor the biological and physiological condition of the human body to support it and to help them cure and diagnose. So, bio-sensors has a very important role in the field of bio-medical engineering to detect the biological effects in form of different electrical signals of physical effects. (Mehrotra P., 2016)

1.2: Biomedical application

Luminescent particles which have high intensity in aggregate form are most appropriate choice of materials to develop fluorescent sensors, organic LED and solid-state lasers.

Whereas light emissive materials have strong intensity in solid phase and weak and light intensity in solution state. So, these types of materials have effect of aggregation caused quenching. These phenomena caused problems in real world when these types of materials used in solid and solution form, where they aggregate.

The development of aggregation induced emission, where aggregation plays key role to emit fluorescence, that enhancing the intensity of light emission. That solves many problems and offer wide use of luminescent materials. These type of AIE materials with adjustable emission colours in solid phase can be used in

various technology application in the area of optical, electronics and bioscience.(H.Tong,2007)

The discovery of new AIEgens , and their mechanism and usefulness of it in advanced technologies will bring this research area to more develop.

Effectiveness of long time cell tracking in a non-invasive is great significance to comprehend beginning, advancement, intrusion, and metastasis of cancer cells. Cell penetrating organic dots with aggregation induced emission (AIE) phenomena is effectively created as long-term cell trackers. The AIE dots appreciate the benefits of high emission efficiency, extensive Stokes move, great biocompatibility, and high photo-stability, which gives their great function in long term non-invasive in vitro cell tracking. In addition, the AIE dots show certain permeability to cell membrane, making them potential choice for cell imaging. The AIE dots show better performance than inorganic quantum dots, opening another area in the improvement of fluorescent tests for observing biological procedures.(Li et al.,2013)

In biomedical application, ACQ is also a mechanism of material that has an emission of light by cell substance. Quenching mostly occurs due to excited state reaction or energy transfer. In quenching, a process occurs in which intensity of fluorescence decreased. So, they become weakly emissive or even non-emissive in condensed phase while they are highly emissive in solid phase. While AIE is luminescent materials that are weakly emissive or even non-emissive in solution but when they aggregate they become highly emissive. So AIE is useful for biomedical research. According to physical fundamental, restriction of molecular movement save energy. In solution, intramolecular rotation occurs, which save energy of excited state non-radiative relaxation. AIE effect occurs with the

restriction of intramolecular motion (RIM) that induces restriction of intramolecular rotation and vibration (Mei et al,2015).

1.3: Objectives of thesis

The objectives of this thesis is to find suitable AIE bio-probes that can be use as alternative ways for cell imaging with the advantage that conventional/commercial available dyes do not have. So, the following steps will be conducted in this research.

- AIE fluorogens have different characteristics to stain different cell compartments. To investigate in which intracellular compartment the AIE fluorogens distribute.
- To investigate whether different incubation time with AIE fluorogens will change its staining pattern in cell line.

- Different cells and different AIE fluorogens need different practical conditions to stain cells. To investigate the optimal condition for the incubation of AIE fluorogens and mammalian cells.
- AIE fluorogens have good biocompatibility. All AIE dyes have different biocompatibility with different cells line. To investigate the biocompatibility/ cytotoxicity of the AIE fluorogens to identify the low cytotoxicity ones.

- To test the utilization of the low cytotoxicity AIE fluorogens in real-time live cell imaging.
- To investigate the BSPOTPE retention time in live cells.

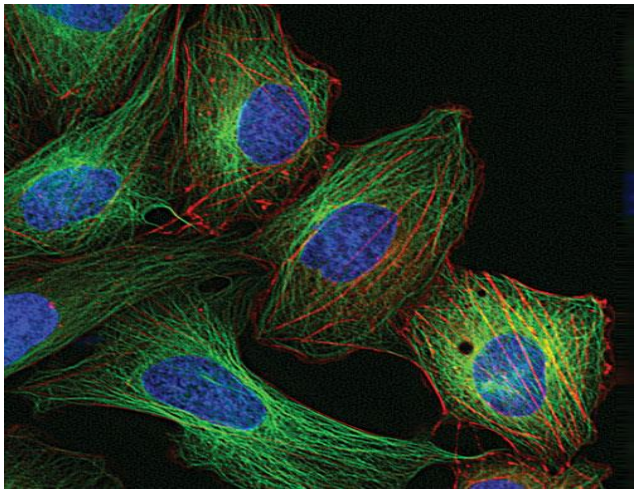


Figure 1.1. Live cell image taken by Incucyte monitor (Zhou et al,2017)

Chapter 2: Literature Review

2.1. Phenomena and mechanism of ACQ and AIE.

In the field of biomedical luminescent used with solution media or in physiological environment, luminescent may show different characteristics depend on their dilution form. When the molecules of sample aggregate together, it may show decreased emission, high emission or no change in emission when diluted. Sometime luminophores have effect of emissive quenching a bit or completely. The luminescent with disc and rod type molecules have π–π interactions. Such aggregates feel delay or no emission on their ground state, and due to that there is quenching effect on luminophores, as shown in Figure 2.1.1 for perylene. In a solid phase it is emissive but as water concentration increases its fluorescent decreased and around the 90% of water emission of fluorescent almost disappear.(Mei et al,2015)

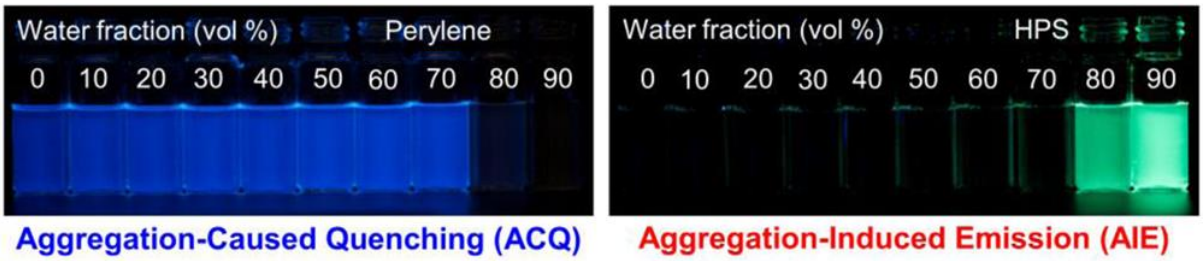


Figure 2.1.1:- (Left) Aggregation-cased quenching effect with perylene and (Right) Aggregation-induced emission with hexaphenylsilole (HPS) .(Mei et al,2015)

In the process of AIE, emission occurs from the non-emissive fluorescent specimen by aggregate formula, as shown in Figure 2.1.1. for hexaphenylsilole (HPS). HPS fluorescent increase as water concentration increase because they aggregate in solution phase.

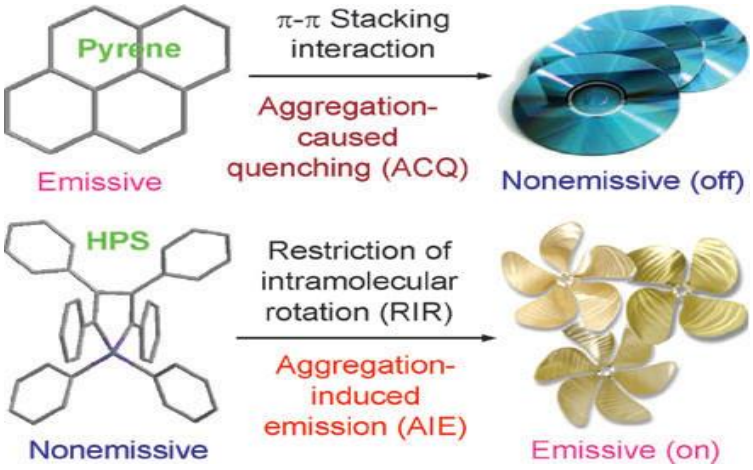


Figure 2.1. 2:- Proposed mechanisms for ACQ and AIE (Hong, Y. (2009).

Figure 2.1.2. shows mechanism of ACQ and AIE fluorescent. ACQ luminophores have disc and rod type molecular structure. In the ACQ there is π–π interaction between disc and rod type molecules and that does not restrict the rotation of the molecules. So, they do not emit light in aggregate form. Because there is no ‘‘solvent’’ in the solid state, the ‘‘solute’ ’molecules are located in the immediate vicinity. The aromatic rings of the neighbouring fluorophores, especially those with disc-like shapes, experience strong π–πstacking interactions, which promotes the formation of aggregates with ordered or random structures. The excited states of the aggregates often decay via non-radiative pathways, which is notoriously known as aggregation-caused quenching (ACQ) of light emission in the condensed phase (Hong, Y. , 2009).

While AIEgens have restriction of intramolecular rotation or motion that restrict the motion of molecules and allow aggregation with each other and they become highly emissive in condensed form.

With the wide research interest for AIE, not just understands the component progressed, but it has advantages of new AIEgens. From pure hydrocarbons to heteroatomic mixes, from little atoms to macromolecules, from natural to inorganic or metallic-natural, the assortment of AIEgens has kept up a consistent

development. For sure, the AIE property is obviously good with other photo-physical impacts like TICT and ESIT. The change from ACQ to AIE can be done by interfacing triphenylamines together with other π bond through single bonds to manage the bond of new AIEgens. The structures of new AIEgens gives various AIE frameworks groups together. Nearly looking at an assortment of new AIEgens will help in the outline of the AIE procedure and ideally inspire something beyond AIEgens.(Mei et al,2015)

Hydrocarbon AIEgens :-

Pure hydrocarbon components remain a vital group of AIEgens for an assortment of reasons. Without the heteroatoms, their properties, e.g., substance, electro, photo-physical, are generally less demanding to study, subsequently giving less difficult system to structure-property relationship investigations and system decipherment. In addition, these frameworks can likewise work as building obstructs for the development of functional AIEgens. Taking TPE as an example, it is the best-known hydro carbonic AIEgen and has been broadly utilized for research, AIE macromolecules development and additionally the ACQ-to-AIE change. The main variables for AIE movement are the motional moieties and auxiliary nonplanarity. In this, with basic hydro carbonic models, this perspective can be further checked and expounded. This area will go over different created immaculate hydrocarbon frameworks, highlighting their basic attributes and revealing the working standards of their AIE impact. (Mei et al, 2015).

2.2. AIE progress as bio-probes in medical research

In the biomedical areas, the luminogens are non-emissive in aqueous buffers but become emissive (or turned on) when bound to biological molecules. Such turn-on biosensors are advantageous over their turn-off counterparts. For example, the turn-on sensors are less likely to generate false-positive signals. They are more sensitive and faster, as the formation of a few emissive aggregates can be readily discerned by the naked eye from the dark background. This is particularly helpful for on-site trials, field screening and household testing, in which constraints in space, transportation, etc. prevent the use of sophisticated instruments. AIE bio-probes actually provide a good platform for biomedical application based on its mechanistic understanding shown in Figure 2.1. 2.

In the past years, there has been done considerable measure of work changing ACQphores into AIEgens that have kept up the useful properties of the regular luminophore and included the advantages of AIE property. The transference of the AIE property into dyes would encourage an immense development in the AIEgenic frameworks, significantly existing AIEgens. This segment has collected a progression of works done on ACQ-to- AIE changes highlighting what has been already done also, what experiences can be picked up. The considerable works have been sorted into the three fundamental groups: (i) enhancing ACQphores with AIE models, (ii) replaceing moieties of AIE models with ACQ units, and (iii) making new AIEgens from ACQphores in view of the RIM rule. (Mei et al,2015)

A most easy way amongst the most approaches to use AIE components into ACQphores is directly joining known AIEgens onto ACQ centres. As an AIEgen itself is electronically conjugated, it can be expected that the merge of ACQ and AIE units at an atomic level would cancel out the ACQ impact and present the

AIE impact on the new luminogen without changing the desired characteristic of the building pieces. (Mei et al,2015) In spite of a few reports with less hopeful results, particular expansion of AIE originals has been useful for most area in the change of numerous traditional luminophores. The achievement of this technique for the ACQ-to-AIE change has been additionally confirmed by the work done on a differing quality of ACQphores. Comparable procedure has additionally been connected to pyrene, an ordinary and usually utilized ACQphore. This experiment was first taken to effectively deracinate the ACQ way of pyrene by symmetrically connecting it with four TPE peripherals to frame the adduct is feebly emissive in the arrangement state and demonstrates a substantial emission improvement in the total or solid state. (Mei et al,2015)

Convert ACQ into AIE:-

Now from all of the effectively changed the ACQphores into AIEgens. In this area, ACQto-AIE changes through measured expansion of AIEgens to ACQphores including anthracene, pyrene, PBI, BODIPY, TPA,

Carbazole have been efficiently expounded with extraordinary consideration paid to the impact of the extent of AIEgens in the AIEgen - ACQphore adducts and their connecting modes on the ACQ-to-AIE change proficiency. The ACQphore -AIEgen adduct may progress toward becoming non-emissive in the solution state if the joined AIE moieties can adequately expend the energized state vitality. At times, if the proportion of the AIEgen to ACQphore is not sufficiently high, the arrangement condition of the adduct may demonstrate emanation to changing degrees. It has been shown that the contorted 3D structure of the prototype AIEgens can disconnect the adducts and obstruct unfavourable intermolecular $\pi-\pi$ stacking connections and in addition the excimer development in the aggregate state; but, the limitation of intramolecular movements would produce results too, which mutually represents the improved emission.(Mei et al,2015) Similarly, the resultant ACQphore- AIEgen adducts are regularly equipped for

incorporating the benefits of the two parts and offering enhanced to a useful collaboration.

Apart from joining AIEgens onto ACQphores, altering prototype AIE cores through the incorporation of ACQphoric units is accepted to be another suitable approach to create new AIEactive frameworks. This technique tries to keep up the current AIE action of the AIEgenic cores while incorporating the useful properties of the ACQphores and taking out their quenching problem.

Basically, the over two procedures for ACQ-to-AIE change are exploiting the components of AIEgens, particularly their dynamic intramolecular movement ability and the 3D nonplanar structures. Since the AIE system and the work specified before, it can be reasoned that the pith of ACQ-to-AIE change procedures is to present adequate motional components and nonplanarity. In the light of this, it can be expected that any approach that matches this pith ought to be workable. Subsequently, making new AIEgens from ACQphores and non-AIE components is possible. Another arrangement of AIE particles have been created by interfacing aromatic stators and rotors together by means of rotatable single bonds. By methods for such design, in the molecularly broke down express, the excited state energy can be scattered through rotational movements and all the more significantly, while in the total express, these movements will be blocked to coordinate energized state decay through radiative means. Likewise, the nonplanar structures started from the contorting way of the connected fragrant rings may keep the subsequent luminogens from close $\pi-\pi$ stacking and maintain a strategic distance from quenching emission in the aggregate state. (Tong et at.,2007)

In perspective of this, many more issues should be answered before advancing exact guidelines to this substitution technique. For example, in what capacity will the size and the state of ACQphores influence the photo-physical properties of the subsequent luminogens. Also, what sort of impact will the conjugation and supplanting destinations force on the photo-physical practices. Hence, in some sense, what we have talked about here for one thing is to highlight the immense occupations that have as of now been done, for the other, is to encourage more consideration and passion to this exploration subject.

Apart from the system of joining rotatory aromatic rings to ACQphores, single-bonded connecting of ACQphores has additionally been utilized to accomplish ACQ-to-AIE change. Nitryl, benzothiadiazole, phenyl, and carbazole are separately attached, which show the AIE activities. These components are exceptionally fluorescent in their crystalline states however non-emissive in their solution state. From their precious stone structures, it is seen that different aromatic species tilt out of plane concerning each other. The bent nonplanar adaptation shows generally free conjugation between the aromatic moieties. It considers rotational movements to happen when the moieties are unconstrained, at last supporting in the emission quenching in solution state. Conversely, in the solid state, the RIR becomes possibly the most important factor and triggers the emission. By holding these electron-pulling back and electron-giving aromatic moieties together by means of single bonds, AIEgens with orange or even red emission can have without problem. Bigger aromatic moieties can likewise be separately connected together through single bonds to frame new AIE particles For instance, a fluorene cores and two fluorenone moieties have been reinforced together to yield. They are emissive in the arrangement state, yet with the expansion of water division, emission turns out to be fundamentally lessened until

a basic point where the luminophores start to aggregate. At this basic point, the RIM procedure causes energized state energy to be casual by means of radiative pathways, prompting the addition in emission. (Yuan et al,2014)

AIE dye as quantum Dots:-

Luminescence as a technique is a perfect choice for the applications in biological areas, in the light of its rich advantages such as superior sensitivity, high selectivity, fast response, low background noise, simplicity, and many others (Hong,2009). A variety of luminescent materials, including organic dyes, quantum dots (QDs), inorganic or organic nanoparticles (NPs), and fluorescent proteins (FPs) have been developed and utilized in biological sensory and imaging applications (Hong,2009). Most of the conventional luminophores suffer from the ACQ effect, which prohibits them from being used at high concentrations and further results in compromised sensitivity.

The conventional luminophores are usually disc-like flat molecules that can emit efficiently when molecularly dissolved or dispersed in their good solvents. However, in the aggregate state, such molecules undergo compact packing and thus strong π−π stacking interactions, further leading to the undesirable ACQ effect. There is indeed vast selection of conventional luminophores. The mature research in the luminescence area has evidenced that many of these conventional dyes have valuable properties, but it is unfortunate that most of them can hardly get rid of the ACQ properties. The ACQ effect has been proven to be a thorny problem that is difficult to tackle. (Hong et al.,2009)

Various traditional strategies were used to solve the ACQ issue but always brought about undesired side effects that do harm to the useful properties of the luminophores. A win-win situation where the ACQ effect is eradicated and

meanwhile the functional properties of the luminogenic molecule are completely retained is hence of inestimable significance and highly desirable.

Diametrically opposed to these conventional luminogens, AIEgens are nearly non-luminescent in the isolated state but luminesce strongly in the aggregate/clustered state. Light-up/ turn-on probes could be facilely engineered and they possess higher resistance to photobleaching and superior reliability relative to those conventional turn-off probes. Moreover, the low background is another merit of AIE-based bio probes, which makes them favourite options for continuous monitoring of biological processes with high sensitivities and resolution, dispensing with repeated washing procedures. (Chen et al,2016)

On the basis of our mechanistic understanding, it can be concluded that there are two key factors which determine the AIE property, one is the moieties that can undergo active intramolecular motions sufficiently dissipating excited-state energy in the isolated states, and the other is the twisted 3D structures in the aggregate states that effectively prevent detrimental π−π stacking interactions. Thus, it is envisaged that integrating these AIE elements into the ACQ systems may work as an effective rationale to transform ACQ luminophores to AIEgens. Here, similar to AIEgens, ACQ luminophores will be referred to as ACQ phores. (Mei et al.,2014)

The concentration–quenching effect has constrained analysts to think about and use fluorophores as confined single particles in diluted solutions. The utilization of diluted solution, in any case, causes numerous issues. For instance, emission from diluted solution are light and weak, prompting poor sensitivity in fluorescence sensory system, particularly in bioassays of follow measures of biomolecules. The sensitivity can't be improved by utilizing high fluorophore focus due to quenching effect. The little quantities of the dye particles in weaken

solution can be immediately photobleached at the point when a cruel laser bar is utilized as the excitation light source. The improvement of inorganic quantum spots (QDs) can surmount these weaknesses yet postures current issues, such as disturbance in components, limitation in variety and high cytotoxicity. Indeed, even in diluted solution, quenching effect is still present and can be included. For instance, in a bioassay framework, the little fluorophore atoms may aggregate on the surfaces of the biomacromolecules and group in the hydrophobic depressions or pockets of the collapsing structures. This can increased nearby fluorophore emission and cause the concentration quenching issue. The quenching effect has been a big problem to the advancement of biosensor strips for on location recognition on the grounds that the fluorophore concentration achieves its most elevated an incentive in the solid state.(Chen et.al,2016)

TPE and its compound use as AIE dye in different Biomedical Fields :-

The connection of the non-conjugated gatherings to the aromatic rings is passive and can even be damaging. For instance, the sterically bulky pendant gatherings can seriously wind the adaptations of the luminogens and subsequently deplorably risk their p-electron conjugation. Then again, the non-conjugated encapsulates furthermore, the straightforward grids utilized as a part of the physical procedures are non-emissive and protecting and are subsequently dilatants and boundaries for luminogen thickness and charge transport, separately. The spatial circulation of the luminogen dopants in a doped film experiences transient shakiness: the luminogens scattered in the polymer lattices slowly move together after some time, in the long run shaping substantial aggregates. Albeit different methodologies have been taken to meddle with luminogen accumulation, the endeavours have met with as it were constrained achievement.(Mei et.al,2015) The trouble lies in the way that aggregation is a natural procedure when luminogenic particles are situated in close region in the dense stage. It will be great if system can be produced, in which light emission is

enhanced, as opposed to quenching, by aggregation. This will make life significantly less demanding in light of the fact that the aggregation attempts to our advantage: no hard work should be done to falsely interfere with the exceptionally characteristic procedure of luminophore aggregation.(Mei et.al,2014)

In biological areas, AIEgens are mainly used as probes for the sensing of biogenic species or as stains/contrast agents for the imaging of cells and organisms or they are even integrated with other functions for diagnosis and/or therapy, in view of this, this section will be split into two smaller sections as biomolecular sensing and biological imaging. Fluorescent bio-probes have been routinely used for visualization and tracking of cellular events. A large variety of organic and inorganic, natural and synthetic materials have been utilized as markers or reporters for differentiation of cellular morphology, among which organic fluorophores are dominant, because they are rich in variety and easy to use. Many of the organic fluorophores, however, contain large fused polynuclear rings, which are prone to aggregate when dispersed in aqueous media, suffering from the notorious ACQ problem. AIE materials provide a way out for organic fluorophores in the area of cell imaging. Aggregates of AIEgens, for example, have been successfully used for long-term cellular tracing. Iron oxide AIEgen nanoparticles with core–shell structures as well as efficient fluorescence and strong magnetization have been fabricating and used for cell imaging (Zhou et.al,2013). Recently, a number of new AIEgens have been designed for specific imaging within cells. Intracellular pH is an essential parameter that regulates and controls many cellular behaviours and processes, such as proliferation and apoptosis as well as enzymatic activity and protein degradation. An abnormal variation in pH may result in dysfunction of cellular organelles. Anomalous pH is a sign of many common diseases. Sensing and monitoring pH changes inside living cells therefore count a great deal for studying cellular metabolisms and

gaining deeper understanding of physiological and pathological processes. Techniques based on fluorescence are the most powerful tools for tracking intact and subcellular pH, due to their extremely high sensitivity and unequalled spatiotemporal resolution (Zhou et al.,2013). The existing pH bio-probes, however, are hard to achieve full-range pH sensing. Recently, we have designed a pH-responsive fluorogen called TPE-Cy, which consists of TPE and cyanine (Cy) units (Chen et.al,2013). It exhibits a large Stokes shift (>185 nm) and shows AIE behaviours, overcoming the limitations of conventional ACQ fluorophores. The well-defined reactivity of TPE-Cy with OH – /H + ions enables it to sense pH in a wide range the broadest so far. AIE has been proven to be a powerful approach for sensitive and specific bio sensing. (Zhou et al.,2013).

Taking advantage of the AIE effect, diverse sensing systems can be facilely established for a wide range of analyses varying from small biogenic molecules to bio macromolecules. This section will be a showcase for these multifarious and excellent AIE-based sensing systems, starting with the ones tailored for biogenic small molecules and followed by those for bio macromolecules.

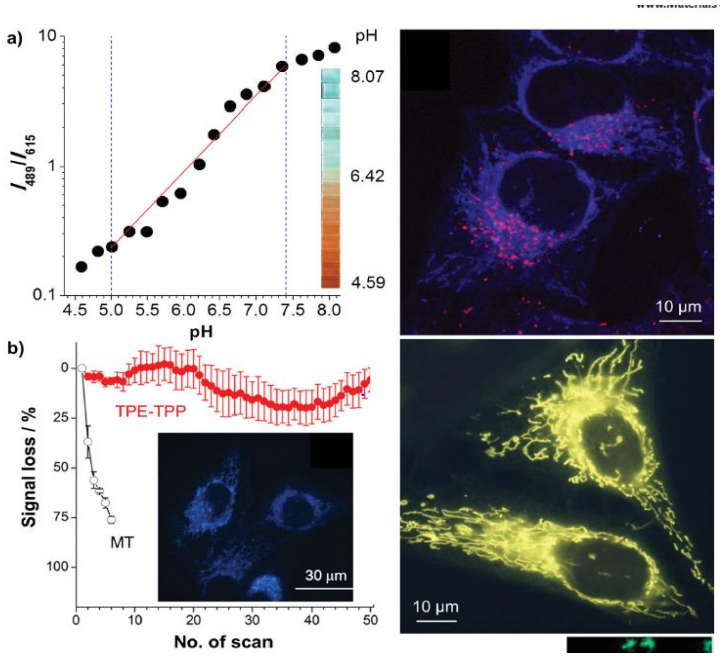


Figure 2.2.1. Cell image and number of scan for TPE-TPP AIEgen (Zhou et al.,2013)

Monitoring the dynamics of mitochondrial morphological changes is attracting increasing attention owing to its involvement in early stage apoptosis and degenerative conditions. To follow the bioprocesses, highly specific and photo-stable bio probes need to be developed. Photo-stable AIEgens for specific mitochondrial imaging have been designed, an example of which is an adduct of TPE and triphenylphosphonium (TPE-TPP) (Zhou et.al,2013). It is prepared by a simple procedure and shows AIE feature with a strong blue emission peaked at 466 nm in solid state. The reticulum structures of mitochondria are clearly visible, thanks to its intense fluorescence. It is photo-stable. The slight fluctuation in the signal may be attributed to the movement of the live cells. In sharp contrast, the signals of the mitochondrial tracker (MT) drop dramatically. When exposed to the excitation light, the outermost layers of the nanoaggregates of TPE-TPP may be photo bleached or photo oxidized but the inner layers are protected from such damages due to dense packing of the particles. Thanks to its high specificity, superb photo stability and great tolerance to microenvironment changes, TPE-TPP is an excellent bio imaging agent for mitochondrial targeting and morphological tracking (Zhou et.al,2013).

Another example of AIEgen-based mitochondrial visualizer is a TPE-pyridinium salt. (Zhao et al.2014) It works as an excellent fluorescent imager for specific staining of mitochondria in living cells with high photo stability(Li et al.,2013). Different from TPE-TPP, is a yellow light emitter, which can afford higher signal-to-noise ratios. It is remarkable that very fine and detailed structures of the mitochondria can be clearly visualized by this brilliant AIE tracker.(Leung et al., 2013) It is well-known that mitochondrion has a large negative potential on the matrix side of membrane. Fluorophore for mitochondrial visualization are thus commonly lipophilic and cationic in character. AIEgenpossesses both of these characteristics and therefore functions as an outstanding staining agent for specific targeting of mitochondria in living cells. Similarly, a conjugated phosphine salt synthesized by Gong, Ning,

et al. has worked well as a fluorescence probe for imaging mitochondria in cells, due to its AIE characteristic, hydrophobic core, and positive charge(Li et al.,2013). Accompanying the advancement in the area of AIE study, a large variety of AIEgens have been developed, which have been used to image almost all the structures in living cells. Thus, in addition to the pH and S-protein, lysosome, cytoplasm and nucleus have all been accomplished.

AIE have different properties like TPE dye recognise bacterial cell by emission of fluorescent. (Zhao et al., 2014) In this research with the help of AIE dye bacterial cell can be seen easily and it also have great biocompatibility so that does not harm other live cells then bacterial cells. AIE dye allows great cell viability and it is easily differentiating from other live cells.

Tumour targeting and imaging are important endeavours in the area of biomedical research. Specially designed AIEgen nanoaggregates have been utilized for various biomedical imaging applications, thanks to their high emission efficiency and superb photo bleaching resistance (Yuan et.al,2014). Fluorescent silica nanoparticles consisting of AIEgen–silica hybrids, for instance, have served as a new platform for the development of multifunctional cellular imaging agents.

Other use of AIEgens dots discovered in this research is it also can be used as blood vessels and capillaries in mouse brain (Li et al., 2015). AIEgens dots also can be used ad chemical probes, gas sensors.

2.3. Commercial available bio-probes for cell images

Bio-probes is used to stain the live or fixed cell. Cell have different parts and different compartment. There are different commercial-available dyes used to stained different compartment and different part of the cells.

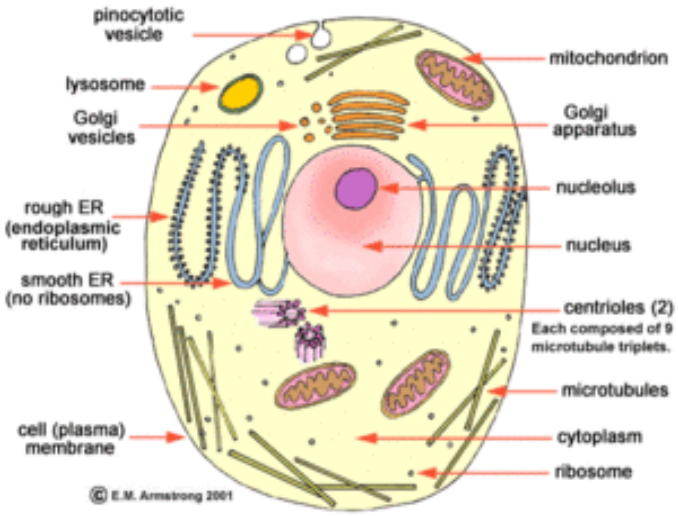


Figure 2.4. Cell structure (net news,2017)

Non-invasive fluorescence cell tracking gives basic data on the physiological displacement, and translocation of effectively migrating cells, which develops our comprehension of biomedical designing, foundational microorganism transplantation and treatments. Non-viral fluorescent protein transfection based cell tracking has been broadly utilized however with issues identified with cell for long time and reliable. Elective cell tracking strategies are in this way sought to achieve dependable, stable, and productive staining for long time. In this work, effectively created organic dots with aggregation induced emission(AIE) and shown their capacities for cell imaging and cell tracking. The AIEgens have high fluorescence, super photo-stability, and great cell retention and biocompatibility. When contrasted with generally utilized pMAX-GFP plasmid marking approach, the organic AIEgens indicated great cell staining on all tried human cell lines and

prevalent quenching, which opens up new area in cell-based immunotherapies and another related biological examines. (Li et al.,2013)

Non-invasive cell marking by organic or inorganic nanomaterials for physiological displacement following has drawn extraordinary consideration now, which is reasonably clear and does not include any changes in the cells. So, accessible fluorescent tests experience the effect of some disadvantages and short tracking time. Like, quantum dots based cell trackers contain toxic substantial metals, while natural particles experience the effects of little Stokes shifts, fast photobleaching and cytoplasm leaking upon cell expansion. Scientists now demonstrated that natural nanoparticles (NPs) have longer cell retention time and decreased exocytosis rate when contrasted with their discrete atomic parts. The aggregation caused quenching effect (ACQ) of commercial organic atoms frequently prompt decreasing or even destroyed fluorescence in NPs. (Ding et.al,2013) A development some fluorogens with aggregation induced emission (AIE) mechanism, which are profoundly emissive when aggregate. In view of these AIE fluorogens, there are effectively developed AIE dots with high fluorescence, great photo-stability and great biocompatibility, uncovering their incredible possibilities to overcome the problems by utilized cell trackers for cell imaging.

2.4. Alternative way of bio-probes for cell imaging by using AIE bio-probes

AIEgens have simple molecular structure and they have low noise in background, highly fluorescent properties and highly biocompatibility and have good photo stability. The advantages of AIE dyes makes them good biological sensors for clinical applications and biomedical imaging of cells. For cancer cell diagnosis and medical treatments, fluorescent dyes have wide range of use.

Long term use and biocompatibility is very important for live cells for tumour imaging and cancer cells response to treatment. So AIE fluorescent dyes are great choice as bio cell trackers for cell imaging because of their properties.

In conclusion, AIEgens have wide range of use and with the high tech innovation offers new opportunity. With its unique properties and different structure, AIEgens get explore their use in cell imaging.

Natural fluorophore-stacked nanoparticles (NPs) have is developed as another era of nanoprobes for bioimaging. For functional biomedical applications, very emissive. NPs are attractive to get high complexity imaging. In a perfect world, the clearest technique to upgrade fluorescence signals is to build the quantity of embodied dyes atoms in every NP. Notwithstanding, there are a few inborn impediments that gives maximum capacity of customary natural dye atoms as delicate fluorescence tests. In the first place, numerous traditional natural fluorophores have hydrophobic planar structures which appear solid intermolecular π–π interactions to support dyes aggregation, prompting quenching fluorescence at high fixations or in aggregation form. Second, the commercial dyes self-absorption coming about because of the little Stokes move of under 25 nm. The poor partition between the absorption and emission spectra additionally show the utilization of optical filters to successfully square excitation light from achieving the fluorescence indicator. Besides, low resistance to photobleaching is another disadvantage of most natural dyes because of photon-incited substance change, which decreased picture quality. The joined disadvantages make commercial dyes not perfect to use in nanoparticle based tests.(Xiao et al.,2016)

Chapter 3: Materials and Method

3.1 : Materials

All the chemicals used in this thesis and project were buy from Sigma-Aldrich, Australia. DMEM as complete growth media, Penicillin-streptomycin as anti-biotics, AIE flurogen BSPOTPE and other commercial AIE flurogen dyes like Vibrant DID, Sir DNA-kit and Sir Tubulin-Kit were also obtained in Australia. BSPOTPE was synthesized according a protocol method and dissolved in Milli-Q water to make 5 mM stock solution. (Zhou et al 2017)

3.2: Cell line and cell culture

HEK 293are human embryonic kidney cells that is used for this thesis experiments as cell line to obtain different results by using AIE flurogen BSPOTPE and other commercial dyes as bio-probes were regularly culturing in complete growth media containing DMEM with 10% fetal bovine serum (FBS) and penicillin and streptomycin is added as antibiotics to protect cell from contaminating with other bacteria and place that in a 5% CO_2 incubator at 37 °C. There is protocol for thaw cells from frozen and protocol for doing subculture of cells, that is as follow:-

Thawing cells from liquid nitrogen

1. Place the vial of frozen cells in a 37°C water bath to thaw within 1-2 min. Sterilize the outside of the vial with 75% Ethanol.
2. Add 1 ml complete growth medium (prewarmed to 37°C) to the tube. Transfer mixture to a 15 ml tube.
3. Add 4 ml complete growth medium and mix gently.
4. Centrifuge at 800 rpm for 5 min. Remove supernatant.
5. Gently resuspend cells in 5 ml complete growth medium.
6. Transfer to a T-25 flask culture at 37°C, 5% CO_2.

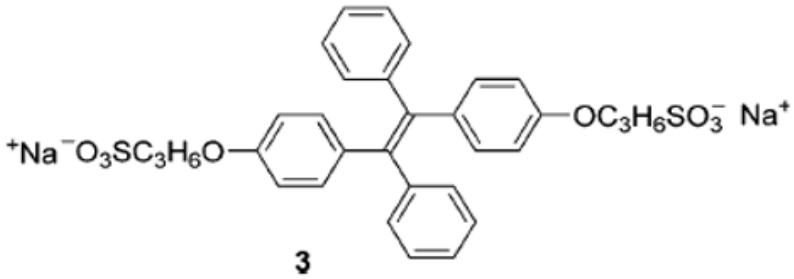
Sub-culturing

7. Aspirate/remove the medium from the flask.
8. Wash the cells once carefully with 5 ml 1XPBS to remove residual medium.
9. Add 1 ml of Trypsin Solution incubate at 37°C until cells have detached (less than 5 minutes).
10. Add 4 ml of complete growth medium to stop trypsin reaction and transfer to a 15 ml centrifuge tube to spin at 800 rpm for 5 min.
11. Remove the supernatant and gently resuspend the cells in 5 ml complete growth medium
12. Transfer 0.5 -1 ml of the above suspension to a new flask with 5 ml fresh complete growth medium. Typically, when HEK 293 cells are confluent, split in a 1:6-1:10 ratio.
13. Cell will become confluent again after 2-3 days.

HEK 293 cells are regularly subculture in every 3-4 days and changing growth media after every 2 days.

3.3: Cell staining by use of Fluorescent dye

For this research BSPOTPE dye is used to stained the cells. As BSPOTPE is desirable choice to use with live cells.



3

Fig 3.3.1: Chemical Structure of BSPOTPE(Tong et al.,2007)

$2X10^5$ cells were seeded in 35 mm petri dish with coverslip to stained the cells with BSPOTPE in buffer solution(HBSS). Then cells were cultured in 5% CO_2 incubator at 37 °C for 24 hrs. Then after 24 hrs, medium was removed from cells and cells were washed 1 time with HBSS. Then after 1ml of BSPOTPE at 200 μM in HBSS added to petri dish and place in incubator with 5% CO_2at 37 °C for 1 hr then replaced with complete growth media.

For cells staining with BSPOTPE in PBS, in 35 mm petri dish with coverslip in it $2X10^5$ cells were seeded then place dish in incubator with 5% CO_2 at 37 °C for 24 hrs. After 24 hrs medium was removed ad wash the cells with PBS for one time, then 1ml of BSPOTPE at 200 μM in PBS were added and incubate the cells for 1 hr. After required time BSPOTPE solution was replaced by complete growth media.

To check the difference in staining pattern of BSPOTPE depend on the different incubation time $2X10^5$ cells were seeded in 3 different 35 mm petri dish then place dishes in incubator with 5% CO_2 at 37 °C for 24 hrs. after 24 hrs cells were stained with 1 ml of BSPOTPE at 200 μM in HBSS for 0.5 hr,1 hr and 2 hrs

respectively then after required time BSPOTPE solution were replaced with growth media.

For co-staining of HEK 293 cells with BSPOTPE and other commercial dyes, first cells were stained with commercial dye to know the pattern of the staining of that dye then HEK 293 were stained with BSPOTPE with other dyes.

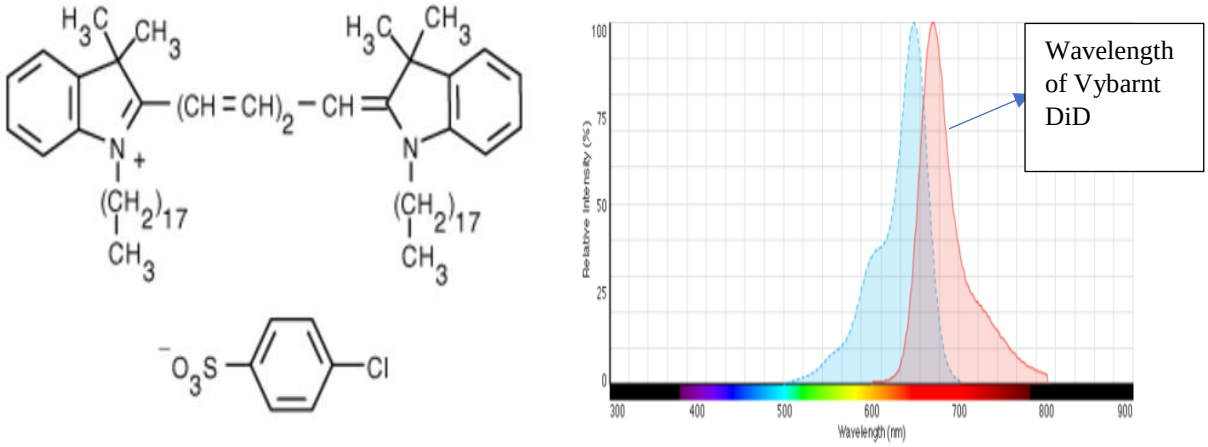


Fig 3.3.2 : Chemical Structure and emission wavelength spetra of Vybrant DiD(Thermofisher.com, 2017).

For BSPOTPE and Vybrant DiD co-staining, 1 ml of growth media containing Vibrant DiD at 5 μM was added to a 35 mm dish with $2X10^5$ cells (seeded 24 h before) and incubated for 0.5 hr. Then Vybrant DiD solution removed and wash the cells with HBSS for 1 time and 1ml of BSPOTPE at 200 μM in HBSS added and incubated for 1 hr in in 5% CO_2 incubator at 37 °C. BSPOTPE was removed and growth media was added after 1 hr.

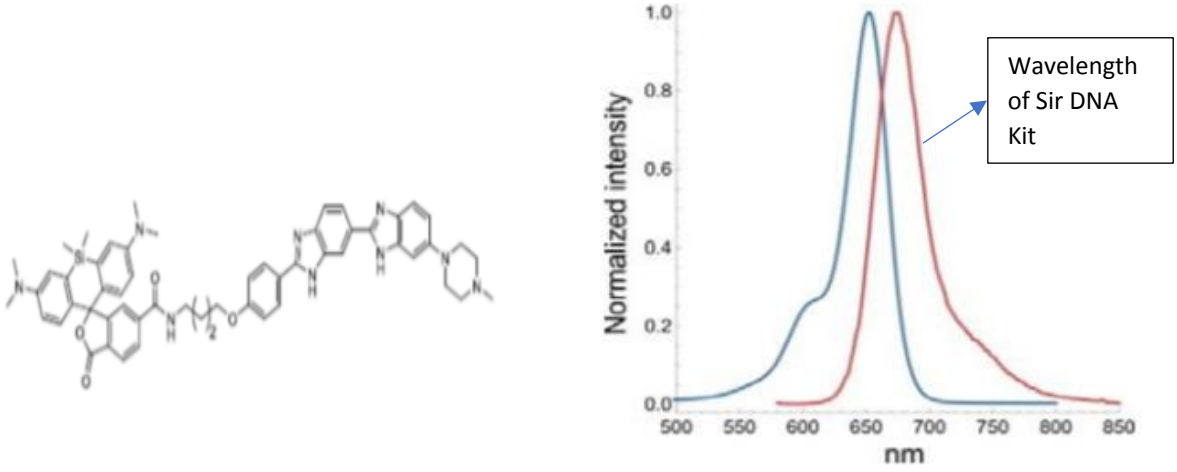


Fig 3.3.3: Chemical structure and emission wavelength spectra of Sir DNA-Kit(Cytoskeleton.com, 2017) .

For BSPOTPE and Sir DNA-kit co-staining, 1 ml of growth media containing Sir DNA-kit at 1 µMconcentration was added to a 35 mm dish with $2X10^5$ cells (seeded 24 h before) and incubated for 0.5 hr. Then Sir DNA-Kit solution removed and wash the cells with HBSS for 1 time and 1ml of BSPOTPE at 200 µM in HBSS added and incubated for 1 hr in in 5% CO_2 incubator at 37 °C. BSPOTPE was removed and growth media was added after 1 hr.

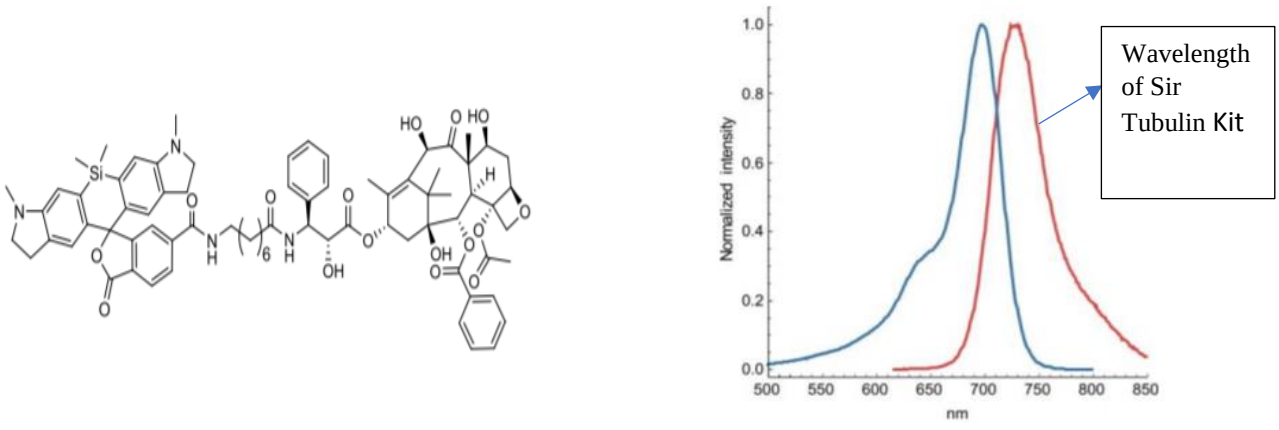


Fig 3.3.4: Chemical Structure and emission wavelength spectra of Sir Tubulin-Kit (Cytoskeleton.com, 2017)

For BSPOTPE and Sir Tubulin-kit co-staining, 1 ml of growth media containing Sir Tubulin-kit at 1 μMconcentration was added to a 35 mm dish with $2X10^5$ cells (seeded 24 h before) and incubated for 0.5 hr. Then Sir Tubulin-Kit solution removed and wash the cells with HBSS for 1 time and 1 ml of BSPOTPE at 200 μM in HBSS added and incubated for 1 hr in in 5% CO_2 incubator at 37 °C. BSPOTPE was removed and growth media was added after 1 hr.

For BSPOTPE retention time in HEK 293 cell's cytoplasm, cells were seeded and stained with BSPOTPE in HBSS with same protocol as above then after staining images of cells were taken using confocal microscopy then cells were again put in petri dish containing growth media with addition of antibiotics and perform the confocal microscopy after 24 hrs , 48 hrs and 72 hrs.

For biocompatibility experiment of BSPOTPE, IncuCyte incubator was used. $2X10^5$ cells were seeded in 24 wall plate before 24 hrs. after 24 hrs cells were stained in different pattern to check the biocompatibility as below:

Column	**1**	**2**	**3**	**4**	**5**	**6**

Column 1: only Growth media added.(no staining, no HBSS)

Column 2:250 ul of HBSS added for 1 hr. then added media(no BESPOTPE)

Column 3: 2:250 ul of HBSS added for 0.5 hr. then added media(no BESPOTPE)

Column 4: 200 uM BESPOTPE added with 500ul media(no HBSS)

Column 5: stained with BESPOTPE in 250 ul HBSS for 1 hr. then added 200uM BESPOTPE in 500ul media added

Column 6:stained with BESPOTPE in 250 ul HBSS for 0.5 hr. then added

for the bio-compatibility Incyucte incubator is used to took the real time images of the live cells. Incyucte incubator is programmed as it took the cell images from all the walls from left to right column every 2 hrs from different 9 position from each wall for 72 hrs. And in this incubator, it provides suitable environment for cells with 5% CO_2 at 37 °C.

Confocal microscopy

All the confocal microscopy was carried out using Leica TCS SP5 scanning confocal microscope at Flinders Microscopy and Biomedical Services, Flinders University. For BSPOTPE fluorescence imaging, 63x water immersion objective lens was used. Diode laser was used to excite BSPOTPE with excitation wavelength = 405 nm, emission was detected using photomultiplier tube (PMT) detector set to detect emission wavelength bandwidth between 410-500 nm.

For the BSPOTPE experiments smart gain was set at 860° and smart off-set was set at -0.4%. while zooming was adjustable and depend on the cell and its place.

For other commercial Far-red dyes, smart gain was set at 960° and smart off-set was set at -0.4% and zooming was adjustable.

For Vybrant DiD detection, 63x water immersion objective lens was used, HeNe laser was used to excite with wavelength= 633 nm. PMT detector was set to detect emission wavelength bandwidth between 650-750 nm.

For Sir DNA-kit detection, 63x water immersion objective lens was used, HeNe laser was used to excite with wavelength= 633 nm. PMT detector was set to detect emission wavelength bandwidth between 650-750 nm.

For Sir Tubulin-kit detection, 63x water immersion objective lens was used, HeNe laser was used to excite with wavelength= 633 nm. PMT detector was set to detect emission wavelength bandwidth between 650-750 nm.

Chapter 4: Results and Discussion

In the research of cell based analysis, fluorescent dyes are very helpful for cell imaging. In this research paper BSPOTPE aggregation induced emission dye is used to stained live cells.

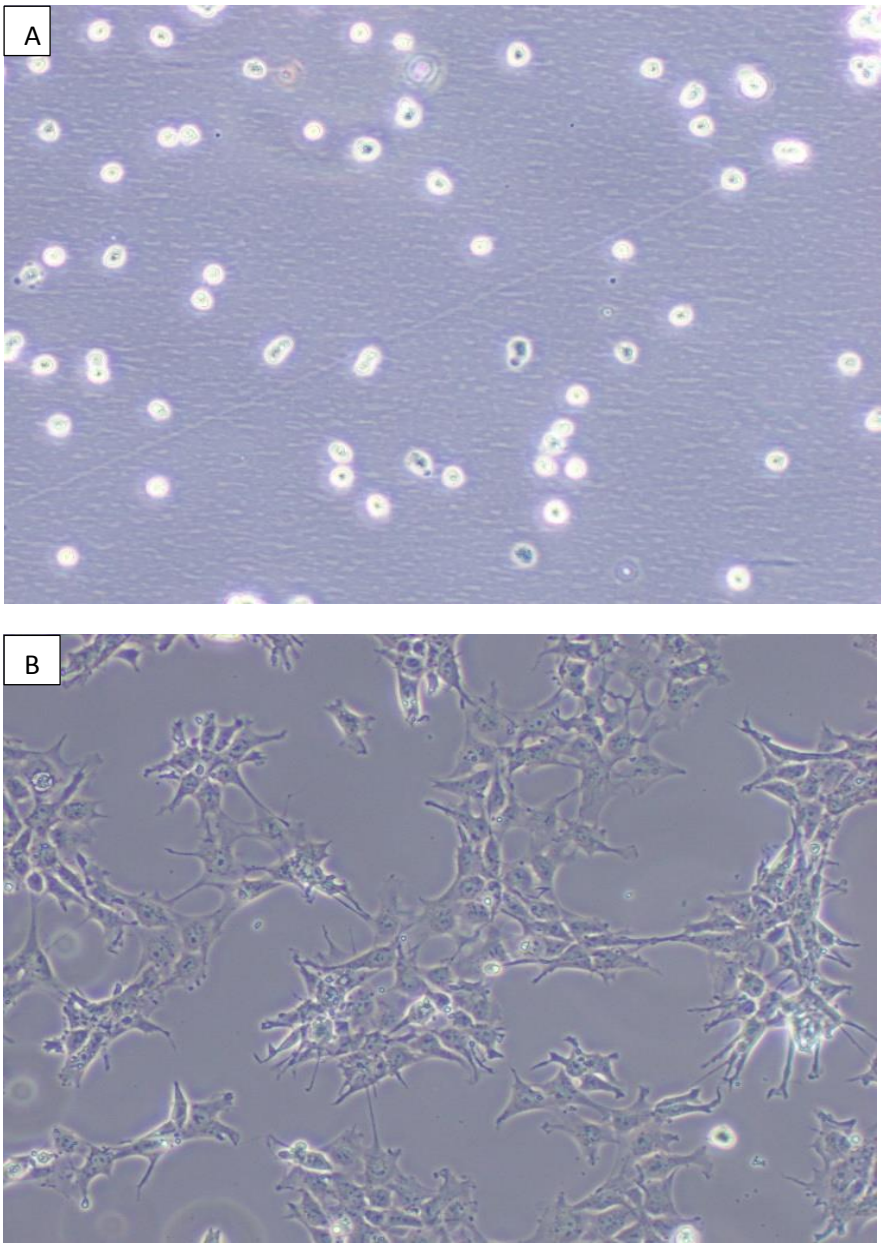


Fig 4.1: cell images taken with 10x magnification with use of microscope.

(A) cell just seeded (B) cell seeded after 24 hrs. grown cells. (in image B cells have increased by number and attached with each other than in image A)

HEK 293 are human embryonic kidney cells. They are faster growing cells. The first image was taken immediately after seeding the cells by using 10x magnification microscope. After that cells were put in incubator at 37 °C with supply of 5% CO_2 and then after next image was taken after 24 hrs using 10x magnification microscope. we can see that the cells were grown properly.

Mammalian cells are very important and useful for research in medical field. Fluorescent dyes are useful to know the activity of cells, cell parts and their response to different treatment and medicines. In cancer research, in vivo imaging fluorescent dyes are very useful. There are different dyes can be used with live cells but they have large emissive wavelength and they cannot use for multiple purpose. But blue fluorescent dye without that large emissive wavelength is useful for many purposes.

4.1 Staining pattern of AIE dyes in HEK293 cells

Before every experiment, one blank cell images are taken that are used for the referencing. To adjust and cancel auto fluorescence from cell and lasers. Like in below images we can see there is no fluorescent signal coming from fluorescent channel and in DIC channel as well we can only see cell with no fluorescence presence.

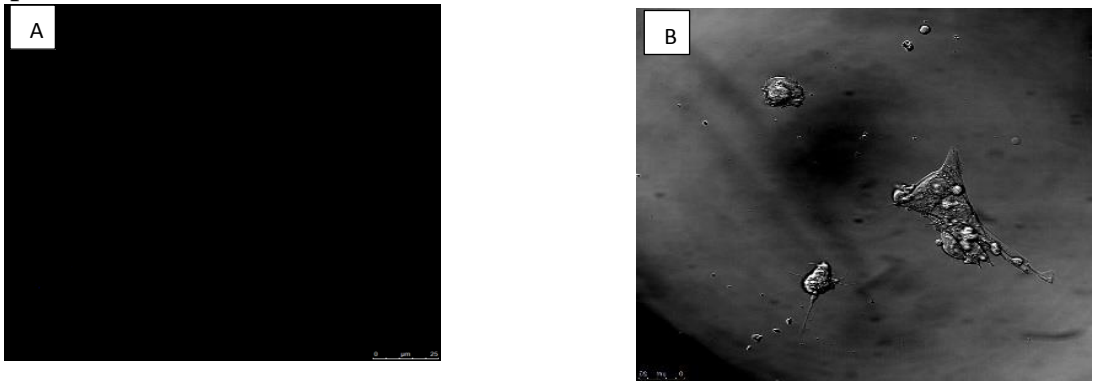


Fig4.1.1: confocal microscopy cell images of blank cells (without any staining) (A) Fluorescent Channel (B) DIC channel

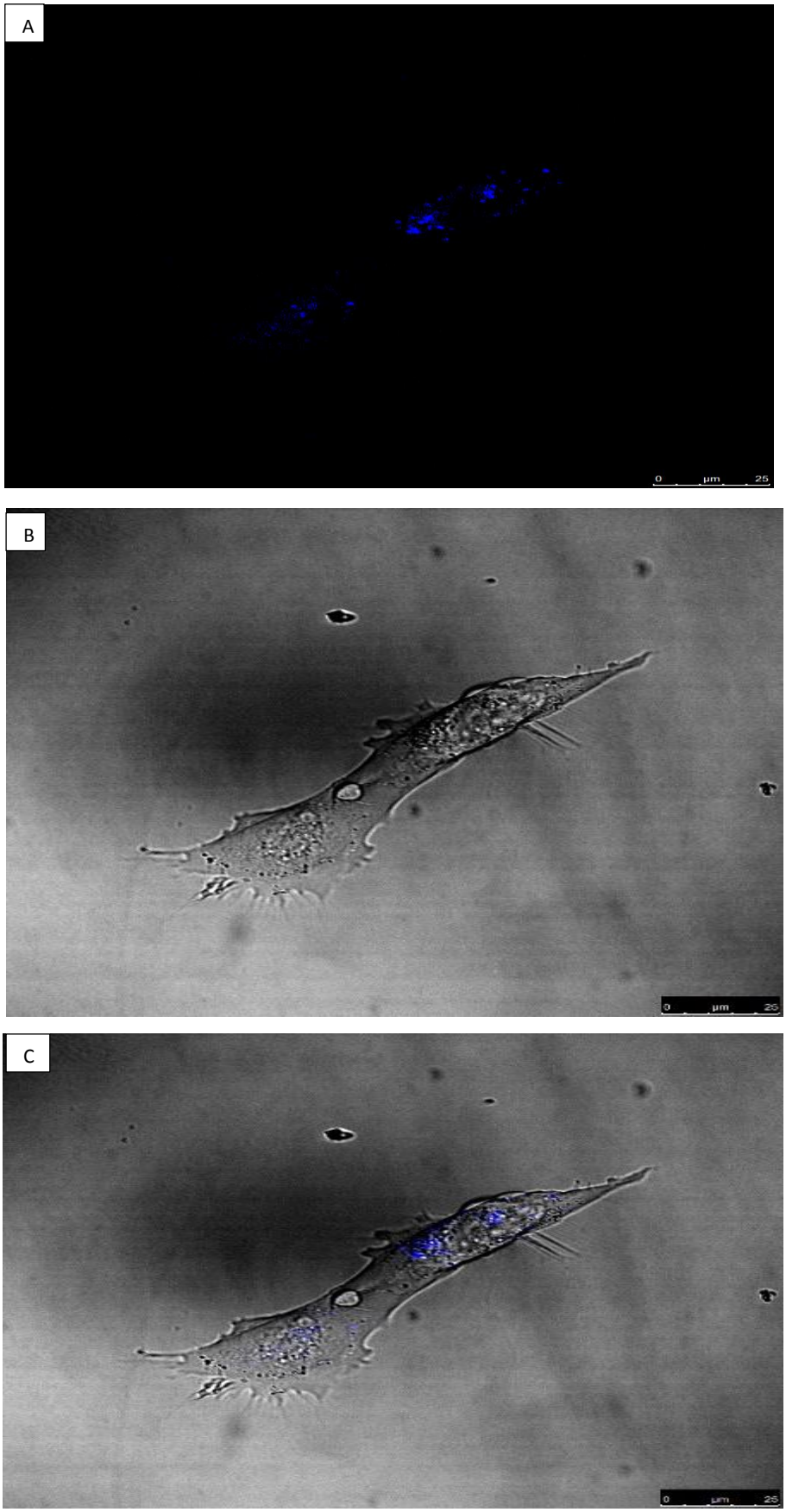


Fig 4.1.2: confocal Microscopy image taken after stain cells with BSPOTPE for 1 hr. (A)BSPOTPE channel (B)DIC channel (c)Overlay channel

BSPOTPE mainly stained cytoplasm of the cells. From fig 4.1.2, we can see that. In DIC channel we can see one clear cell, and in the BSPOTPE channel, cell's cytoplasm is stained by the BSPOTPE. But there is difference of staining pattern depend on the buffer solutions.

Like if, cells are in PBS solution then cells are more stressed then HBSS solution because it contains useful salts. Main difference between stressed cells and normal cells is permeability membrane which permits BSPOTPE to get inside the cells for staining.

BSPOTPE is used mainly for albumin protein binding but it can also use to bind other cellular proteins, like during protein unfolding process the molten globule intermediate. (Hong et al,2009) 1ml of BSPOTPE at 200 μM in PBS, staining pattern change depend on the buffer solution confocal microscopy images shows that in PBS solution BSPOTPE stained cytoplasm and nuclei. In PBS solution shape of cells become round that indicates stress in the cells because PBS does not provide required salts to cell for living, whereas HBSS contains required salts and D-glucose which gives suitable environment to the cells.

The stress in the cells are not good for the healthy cells. It changes many cellular properties and permeability of cell membrane. It changes nuclear membrane that allows to BSPOTPE to pass into cell. When some experiments perform on the fixed cells and adding dyes in to it is not healthy for cells as some of the soluble proteins of cells may be lost in the process of cell fixation, proteins which are bound to cellular organelle and cytoskeleton would present and stay in the cell. Commercial fluorescent dyes that are used in the different fixed cell line can stain normally cytoskeleton proteins or aforementioned proteins like F-actin. But

BSPOTPE is used to bind the proteins and that is suggested that BSPOTPE fluorescence binds subcellular proteins or cytoskeleton. There is no proof of nuclear acid can be bound by BSPOTPE in nucleus. The binding of proteins by BSPOTPE likely to be in the nucleoli.

BSPOTPE is permeable to plasma membrane, after entering to cytoplasm it binds to cellular proteins and stain the cytoplasm. BSPOTPE has high specificity to albumin but during the protein unfolding process it can also be attached with molten globule intermediate. HBSS as buffer solution have various useful salts and D-glucose which give more favourable condition to cells and BSPOTPE to pass through plasma membrane and enters into cytoplasm. (Zhou et al., 2017)

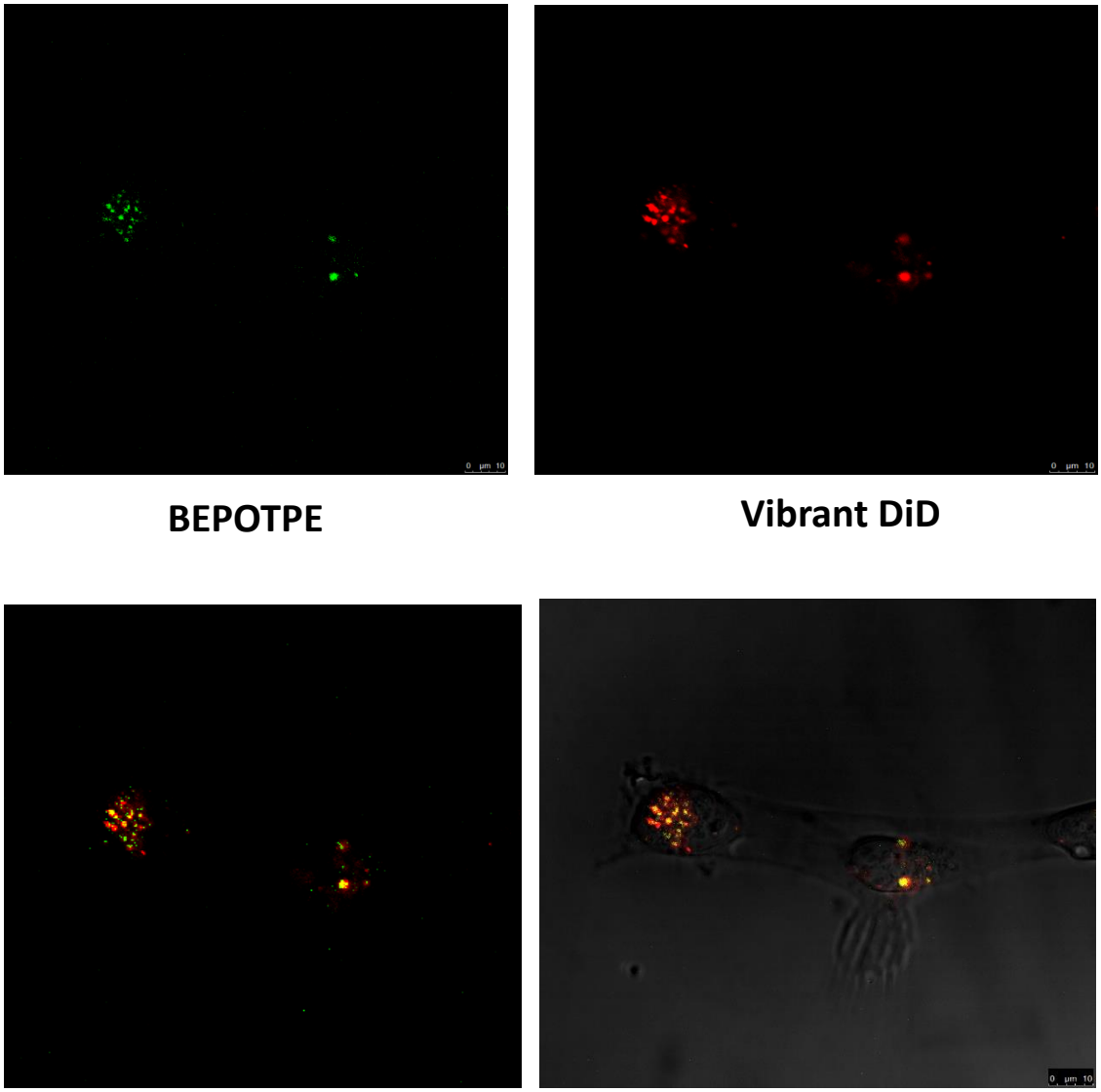


Fig 4.1.3: Confocal images of cell stain with (A) BSPOTPE for 1 hr (B) Vybrant DiD for 0.5 hr (C) Overlay channel of BSPOTPE and Vybrant DiD (D) Overlay channel

BSPOTPE can be used with other far-red emissive wavelength dyes. For example, if BSPOTPE used with Vibrant Did then it can be seen that the part of

cells which are not stained by BSPOTPE that stained by Vibrant Did dye and the part cells which are not stained by Vibrant Did dye that stained by BSPOTPE. So, by this cell structure and their activity can be easily found.Vybrant Did dye has emission wavelength of approximately 670 nm so that does not interfere with BSPOTPE emission wave-length. Vybrant DiD stain cell membrane and intracellular lipids. In the image, green colour used for BSPOTPE instead of its original blue colour to easily differentiate between BSPOTPE and Vybrant DiD with red colour. We can see in confocal microscopy green colour and red colour co-localisation and we can see yellow colour in cells that shows BSPOTPE and Vybrant DiD dye co-localisation in cells. That region shows presences of BSPOTPE in cytoplasm and Vybrant DiD in intracellular lipids.

In the fig 4.1.3 of Vybarnt DiD red staining colour indicates presences of it in plasma membrane and no presences of BSPOTPE and green region indicates presences of BSPOTPE and no Vybrant Did staining. Both dyes co staining images indicates that BSPOTPE does not stain plasm membrane and Vybrant DiD does not stain cytoplasm in the cells.

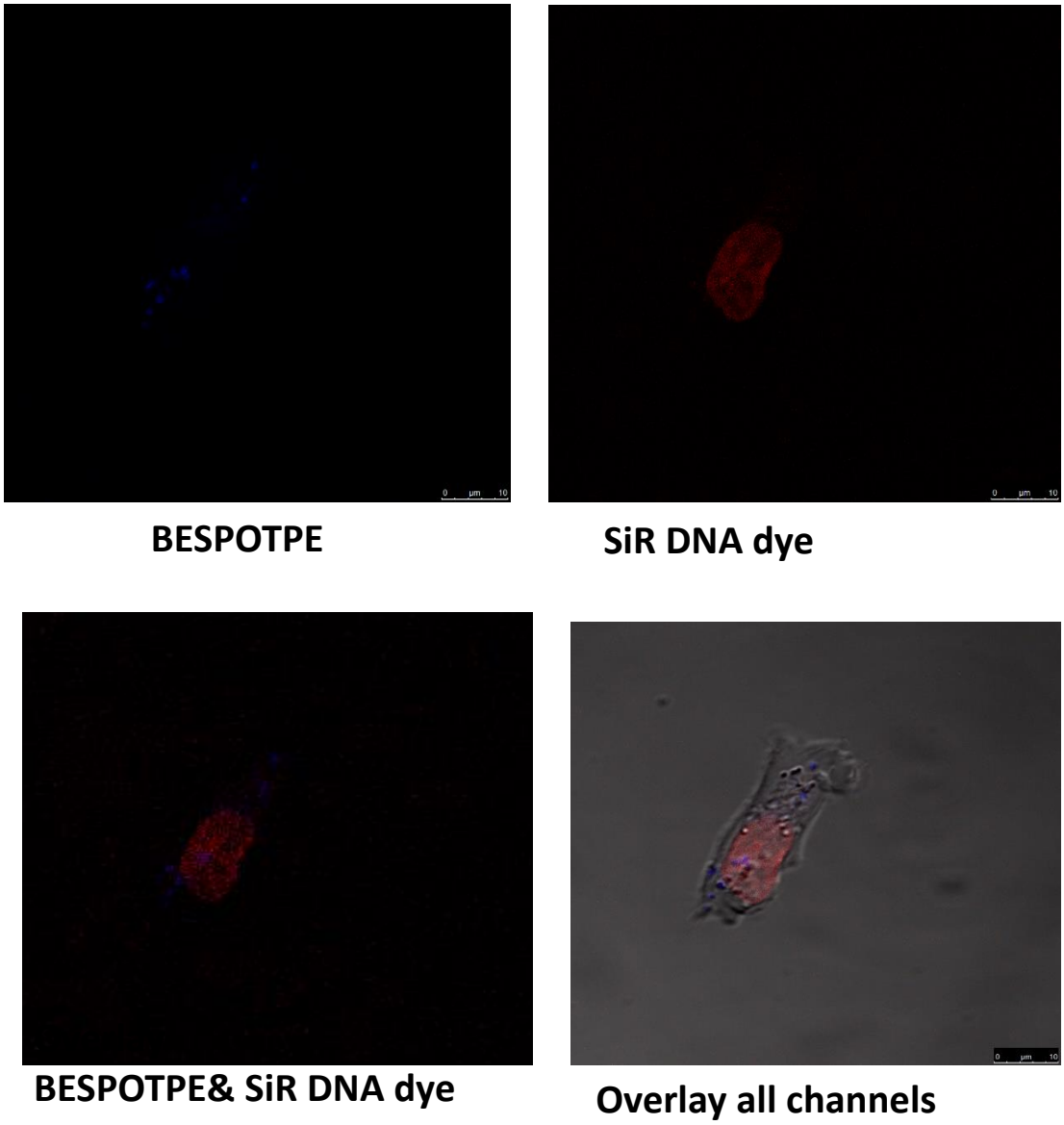


Fig 4.1.4: Confocal images of cell stain with (A) BSPOTPE for 1 hr (B) sir DNA-Kit for 0.5 hr (C) Overlay channel of BSPOTPE and Sir DNA-Kit (D) Overlay channel

HEK 293 cell co-staining with BSPOTPE and Sir-DNA kit commercial dye with the emissive wave length of 674 nm which have far-red emission. From the confocal microscopy images 4.1.4, we can see that Sir DNA kit stain the nucleus of the cell and BSPOTPE stain cytoplasm of cell. In the overlay channel of BSPOTPE and DNA kit we can see co-localisation in the cell that both dyes stain different compartment of the cell and they are not interfering in staining. There is no presence of BSPOTPE in nucleus and there is no presence of Sir DNA kit in cytoplasm.

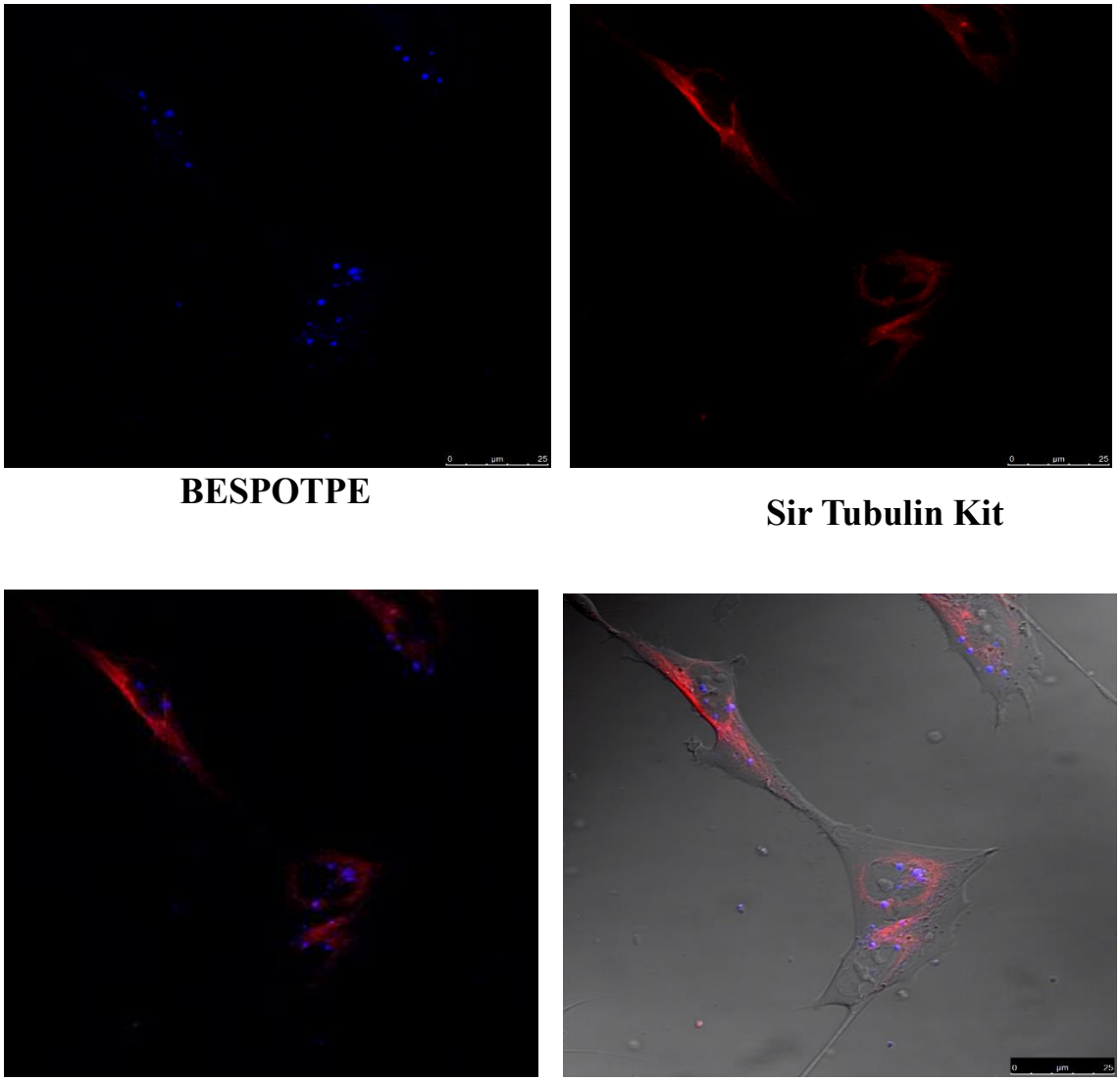
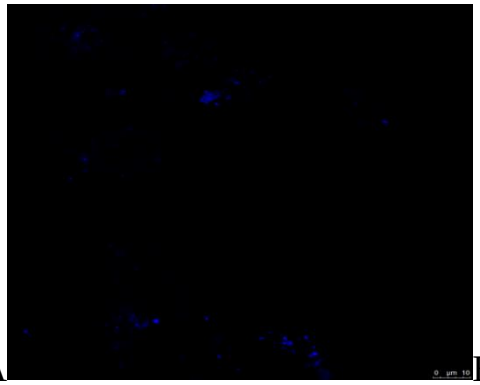
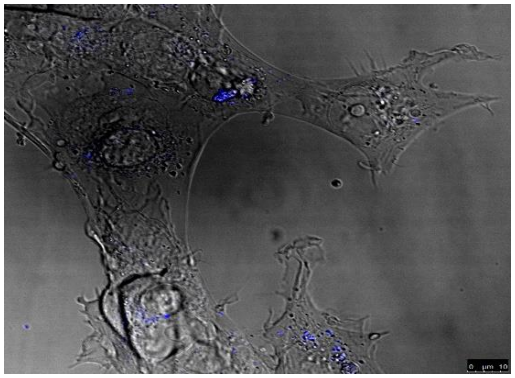


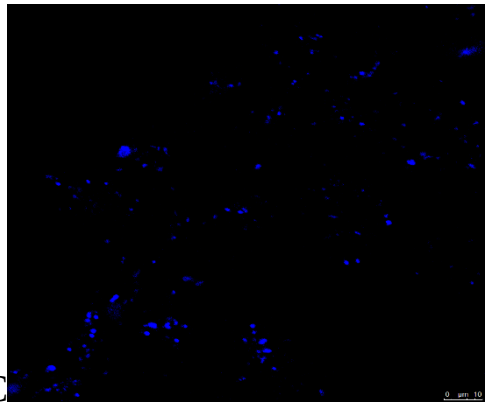
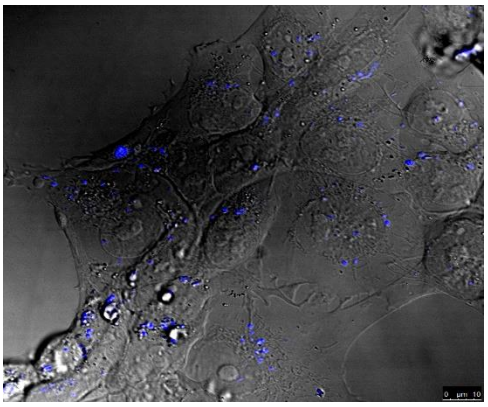
Fig 4.1.5: Confocal images of cell stain with (A) BSPOTPE for 1 hr (B) sir Tubulin-Kit for 0.5 hr (C) Overlay channel of BSPOTPE and Sir Tubulin-Kit (D) Overlay channel

Sir Tubulin kit is cytoskeleton Commercial ALE dye that used to know the cell compartment structure. It has emissive wavelength of 674 nm with far-red emission. Cell co-staining with BSPOTPE and Sir Tubulin kit gives staining of cytoplasm stained by BSPOTPE and SIR Tubulin kit give cytoskeleton structure of HEK293 cells. On the overlay channels we can see the cell compartment's skeleton like structure and cytoplasm of cell that is stained by BSPOTPE. These both dyes do not interfere in each other staining pattern. (fig-4.1.5)

By using different AIE dyes in HEK 293 cells, we can know the different compartment stained by different dyes. From the co-staining of BSPOTPE and commercial AIE dyes in cells we can see that BSPOTPE is not interfering the staining pattern of commercial dyes. By using different commercial dyes we can get images of different cell compartment and know the activity and structure of that particular compartment. By using BSPOTPE we can get images of cytoplasm in cells. Commercial dyes like Vybarnt DiD gives Cytoplasm images, Sir DNA kit stained nucleus of the cells and Sir Tubulin Kit gives cytoskeleton structure of cell and cell compartment.

4.2. Different Incubation time of staining

A  B 

C  D 

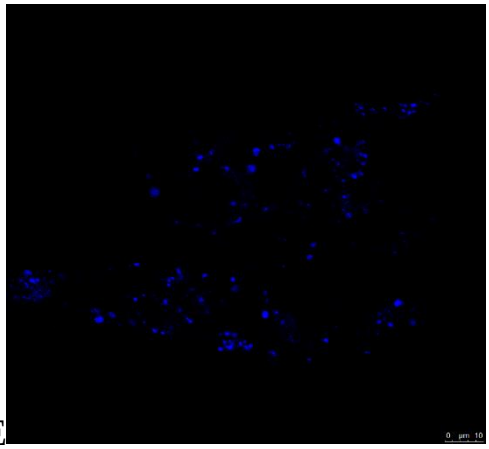
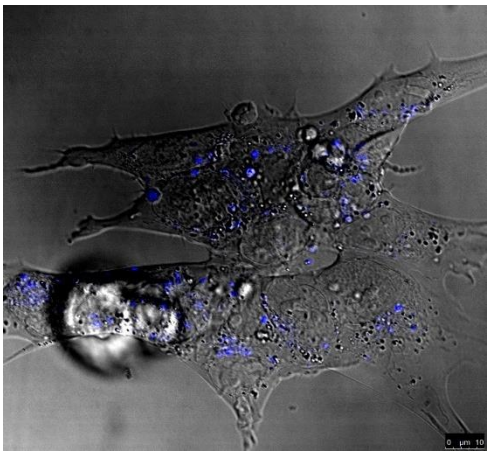
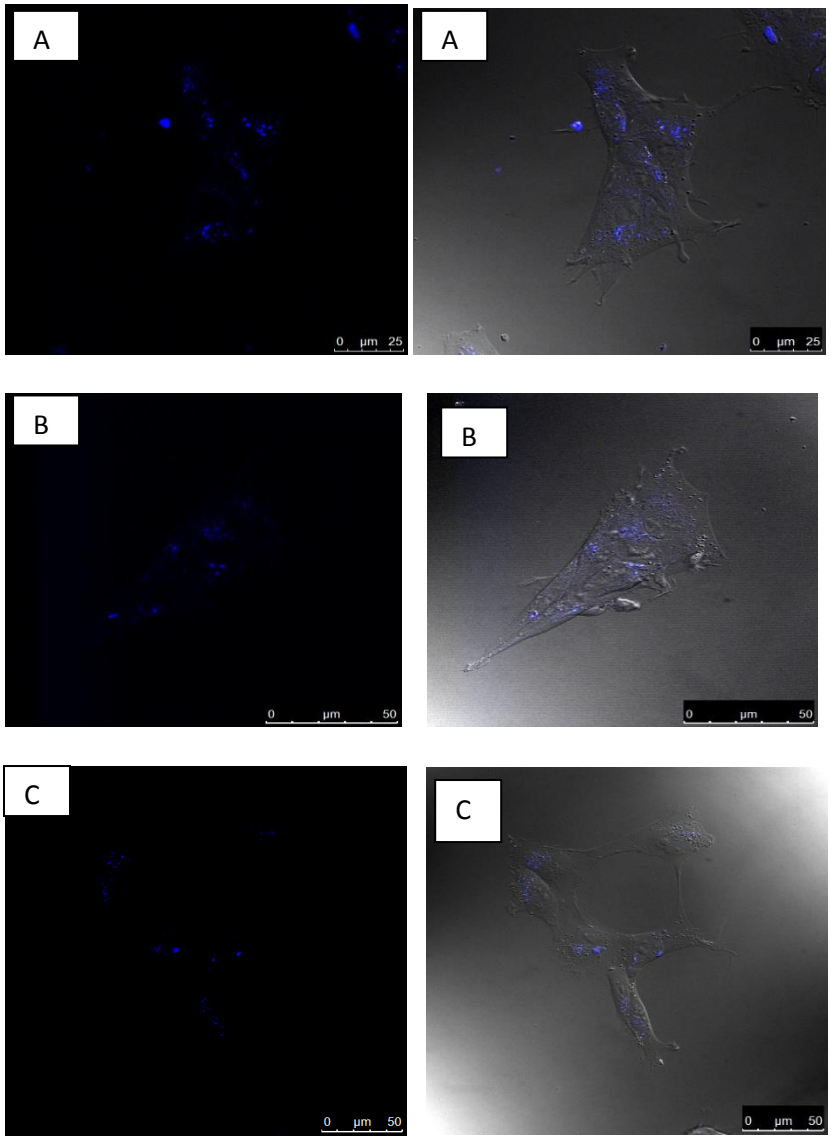
E  F 

Fig 4.2.1: A) BSPOTPE staining for 0.5hr.(B) DIC channel for BSPOTPE staining for 0.5 hr.(C) BSPOTPE staining for 1hr (D) DIC channel for BSPOTPE staining for 1hr (E) BSPOTPE staining for 2 hrs (F) DIC channel for BSPOTPE staining for 2 hrs

In the Experiment, for the staining pattern of the 1ml of BSPOTPE at 200 μM in HEK 293 cells were seeded before 24 hrs and then stained with BSPOTPE in HBSS solution for 3 different times like 0.5 hr, 1hr and 2 hrs respectively. After doing confocal microscopy we can see from fig 4.2.1, that different incubation time does not change the staining pattern of BSPOTPE. The change is just the brightness and intensity of the fluorescent like coming from cell. The experiment tells that BSPOTPE in water solution can permeable in cell membrane, and stain the cell's cytoplasm and binds the cell protein.

4.3 Retention time in cells



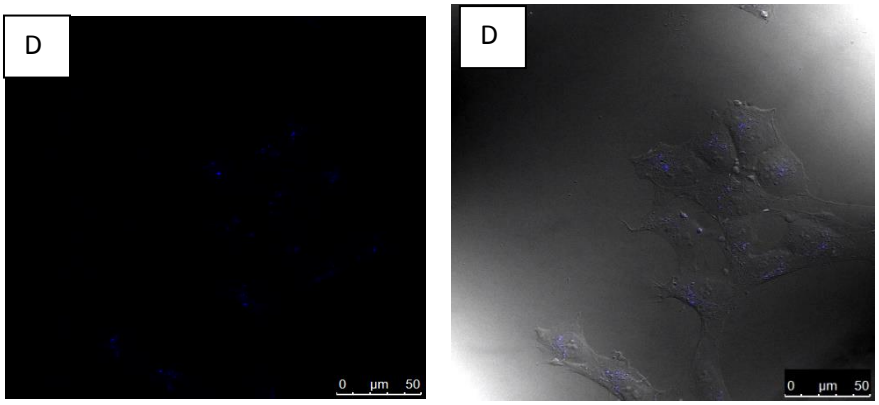


Fig 4.3.1: Confocal image of cells stained with BSPOTPE to see its retention time (A) t = 0 hr (B) t= 24 hrs (C) t= 48 hrs (D) t=72 hrs

BSPOTPE has very good retention time in cell membrane. In our experiment, we can see fluorescent light coming from cells even after 72 hrs thought it is comparatively low from 0 hrs, 24 hrs and 48 hrs. we can see that intensity of fluorescent decreased as time of images taken increase. But we can see that BSPOTPE staining is also passes to the 2^{nd} and 3^{rd} generation daughter cell as they have newly grown as division. After 72 hrs almost 3 generation cells are grown and they all have staining from BSPOTPE. So BSPOTPE staining and permeability in cells is strong enough that it can passed to daughter cells as well.

4.4 Biocompatibility of BSPOTPE

Any substance or material which are compatible with living tissue and does not have any toxic effect, not being injurious to living system and does not cause any physiological reactions and changes that is called biocompatibility.

To check the biocompatibility of the BSPOTPE, HEK293 cells were seeded 24 hrs before staining with BSPOTPE in 24 wall plate. There is different staining

pattern used to stain the cells to check the bio compatibility. The pattern are as follow:

Column 1: only Growth media added.(no staining, no HBSS)

Column 2:250 ul of HBSS added for 1 hr. then added media(no BESPOTPE)

Column 3: 2:250 ul of HBSS added for 0.5 hr. then added media(no BESPOTPE)

Column 4: 200 uM BESPOTPE added with 500ul media(no HBSS)

Column 5: stained with BESPOTPE in 250 ul HBSS for 1 hr. then added 200uM BESPOTPE in 500ul media added

Column 6:stained with BESPOTPE in 250 ul HBSS for 0.5 hr. then added 200uM BESPOTPE in 500ul media added

Then this plate of cells stained with BSPOTPE put in the Incyucte incubator and set the program to take the images of cells from each wall every 2 hours for 72 hrs. And then created a graph from the collected data is as below:

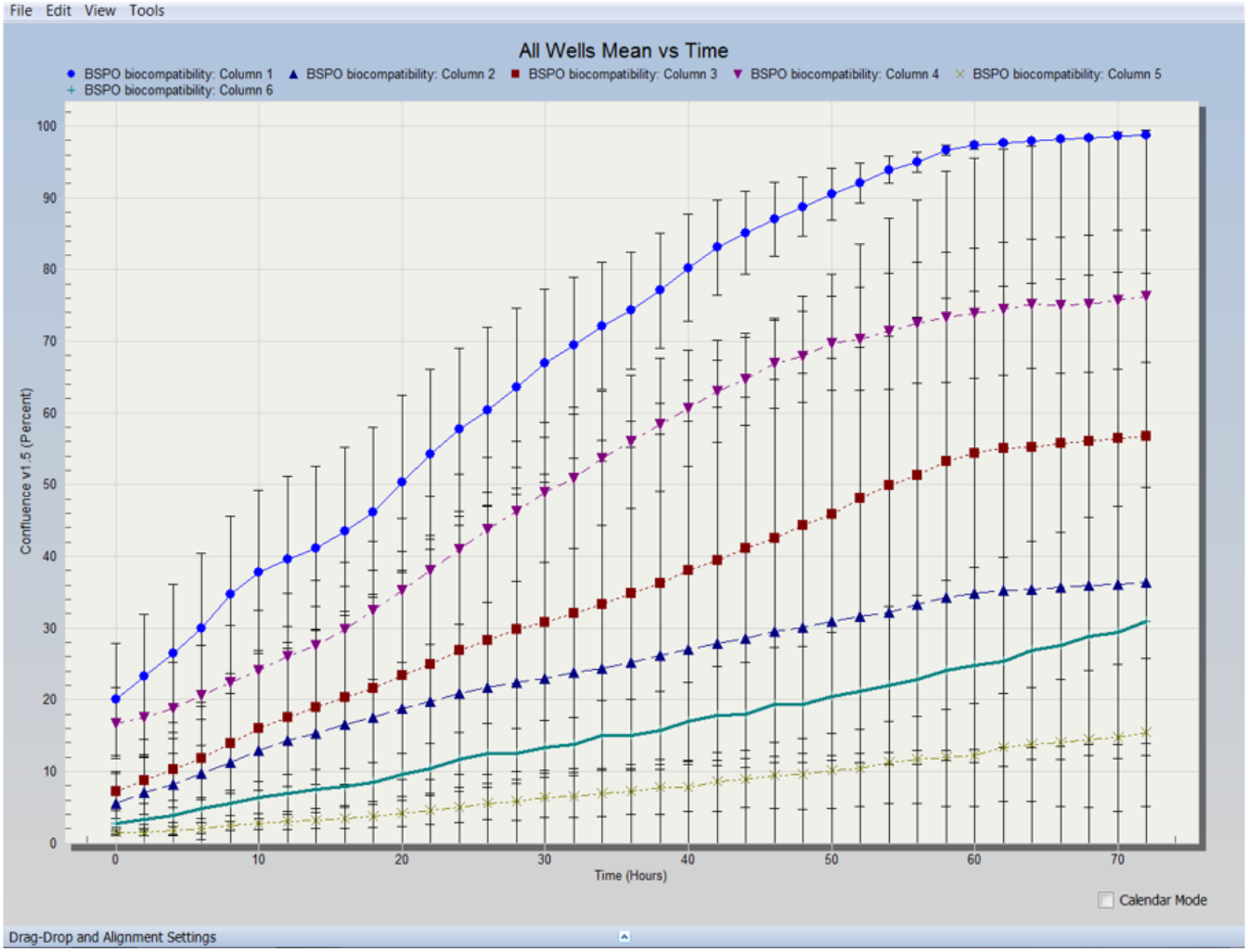


Fig 4.4.1: Real time image taken by IncuCyte monitor of HEK293 cells.

This fig 4.4.1 show growing pattern of the cells. Which is differ from each other. If BSPOTPE is biocompatible then cells will grow and meet at the same 100% confluent point. But in this graph, each column cells have different growing pattern. the first assumption for the reason behind this may be while performing the experiment some of the cell may be washed off. So, their density was different from each other. May be because of this we can see the difference in the growing pattern. in the graph we can see column 1 cells are 100% confluent and fully grown, because that cells were only contain growth media and they are not washed. While column 5 and column 6 which cells contained BSPOTPE in HBSS and then BSPOTPE in growth media were washed twice. So, may be their cells come off while adding two different BSPOTPE solutions.

Other assumption is BSPOTPE may show some cytotoxicity effects in the HEK293 cell line as this is embryonic cells. It may be happened that BSPOTPE may not be bio-compatible for this cell line. As we can see from the fig 4.4.1 that the cell column that only contain growth media are fully grown as compare to another cell column. We can see that in column 5 and column 6 which contained BSPOTPE in two different solutions like in HBSS and in the Growth media that cells were not confluent properly that show some toxic effects of BSPOTPE.

But on the other side, on the other cell line HCT116 colorectal cancer cells BSPOTPE bio-compatibility is checked by other lab member followed by same experimental protocol that results on the graph is shown below:

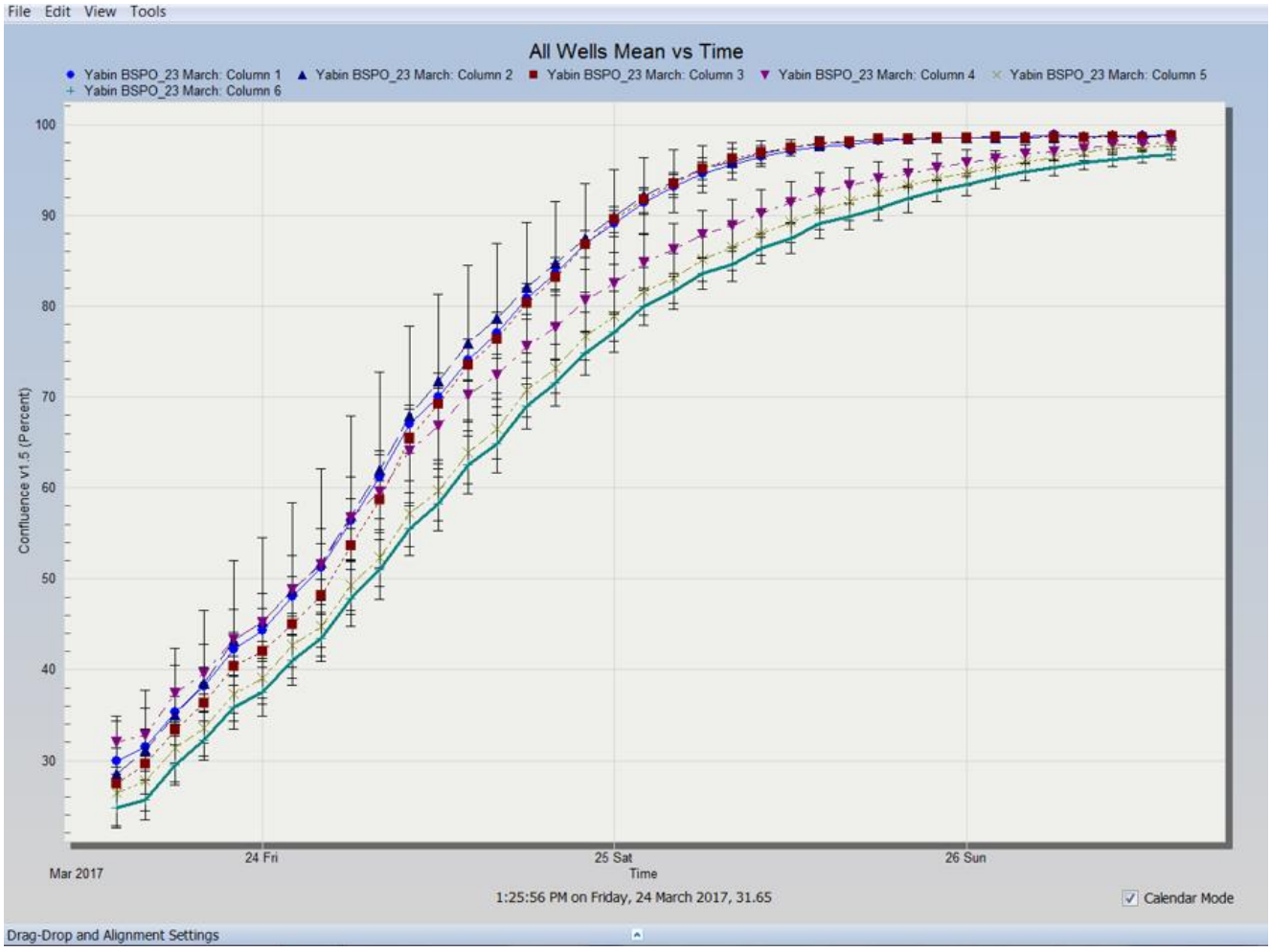


Fig 4.4.2: Real time image taken by IncuCyte Monitor. This experiment performed by other lab member on HCT 116 Cells using same method.

This fig 4.4.2 shows BSPOTPE biocompatibility in the cancer cell line. As all cell column are fully grown and 100% confluent. There is different growing pattern but at the end of 72 hrs all cells are properly grown.

So, from the both the fig 4.4.1 and 4.4.2 we can say that BSPOTPE is bio-compatible for cancer cells. But due to some experimental faults it shows some toxic effects in the embryonic HEK293 normal cells. So, from this for the future researches BSPOTPE biocompatibility in the human embryonic cells can be check and oerfoem experiments on it.

4.5 Photo-stability of BSPOTPE in cells

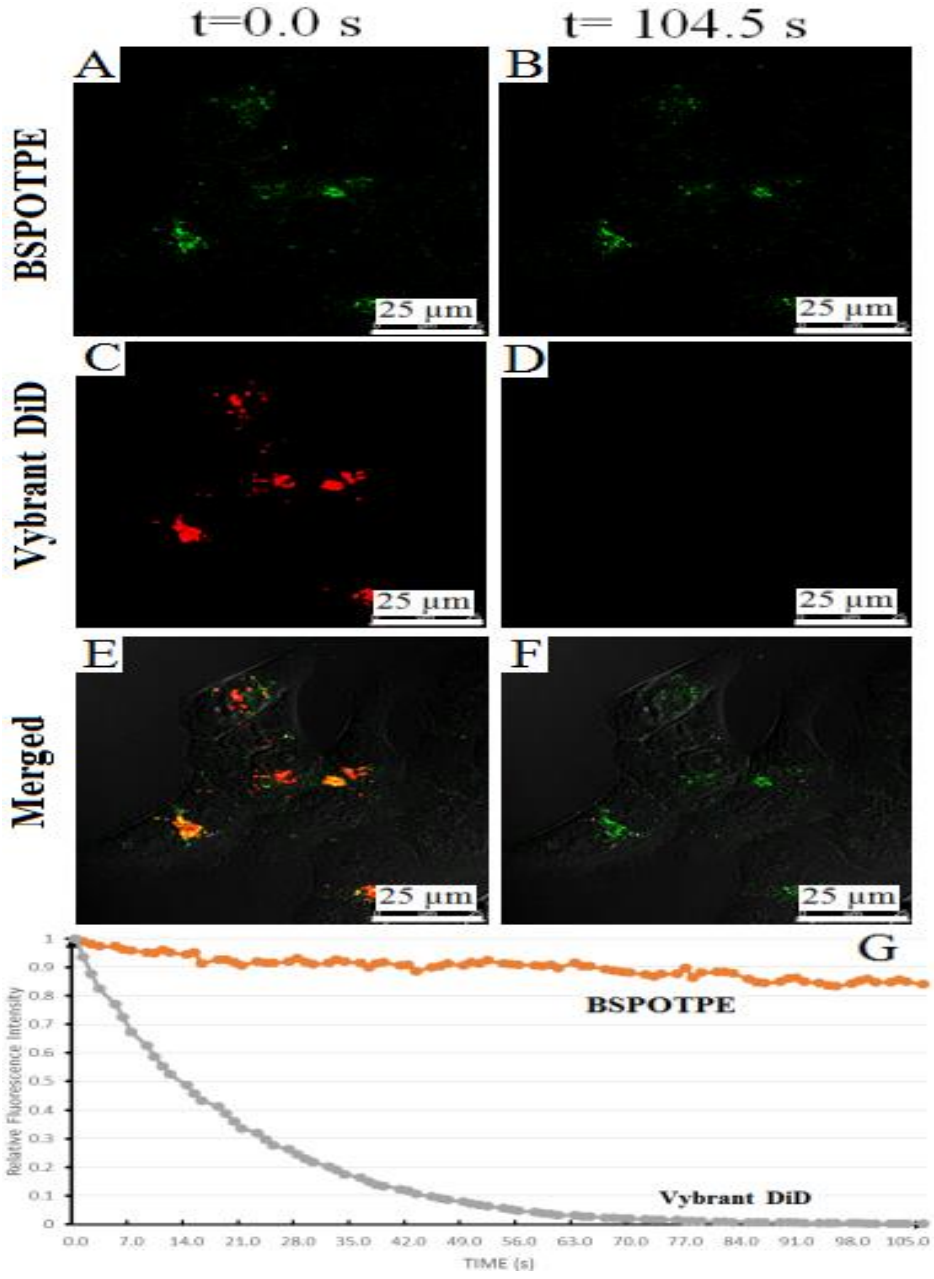


Fig 4.5.1: Confocal analysis of the photo-stability between (**A, C, E**) BSPOTPE and (**B, D, F**) Vybrant DiD and (**G**) relative fluorescent intensity as a function of time for BSPOTPE and Vybrant DiD, showing excellent photo-stability of BSPOTPE.(Zhou et al. ,2017)

It is interesting to check the photostability of BSPOTPE staining in different cell line HCT116 performed by other lab member. It first check with commonly used membrane staining dye Vybrant Did as both dyes have different emission wavelength, experiment was performed as simultaneous scans for both cell at each 1.29 sec till 82 scans. The results shows that BSPOTPE has high photostability then Vybarnt Did, after 104.5 sec BSPOTPE had still higher intensity of fluorescence then Vybrant Did. (Zhou et al., 2017)

Chapter 5: Future Directions

As from the experimental results, we can say that there can be many research will be performed on the AIEgen BSPOTPE. This is wide topic to for researchers. Mainly in future BSPOTPE bio-compatibility experiment can be performed to check whether it has cytotoxic effects on cells or it was just experimental errors. And BSPOTPE photo-stability can be compared with commercial dyes Sir DNA-Kit and Sir Tubulin-Kit. As well as other commercial dyes like Sir Actin-kit could be used to stained the cells, and know which cellular compartment is stained by this dye and how cells react with these dyes.

5.1 : Conclusion

In conclusion, we can show from research that water-soluble AIE fluorogen BSPOTPE specifically stained cytoplasm in healthy HEK293 cells. It has very good photo-stability. It has excellent retention time that last up to 72 hrs, so long time cell tracking experiments can easily carry out. It also passed staining to daughter cells in cell division process. All these properties make BSPOTPE a good choice for live cell staining and tracking. Moreover, blue fluorescence emission of BSPOTPE gives advantages to use with red and green emissive dyes. This dye conjugated with antibiotics and proteins. For live cell tracking BSPOTPE is great choice.

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