ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.2.L1-17

International Journal of Lifescience and Pharma Research

Review Article

Chemistry for New drug discovery

Recent Advancement in Fluorescent Probes for Sensing and Imaging Tyrosinase Activity in Living Cells

Krishanu Sarkar*

Assistant Professor, Department of Chemistry, Netaji Mahavidyalaya, Arambagh, Hooghly, 712601, West Bengal, India

Abstract: Tyrosinase, a multi-copper oxidase enzyme, is widely distributed in different organisms and plays a vital role in the melanogenesis and enzymatic browning in fruits and vegetables. An abnormal level of tyrosinase in the living system is often associated with different kinds of skin diseases including albinism, vitiligo, skin hyperpigmentation etc. Moreover, overexpressed tyrosinase has become a prognostic biomarker for melanoma. Therefore early detection of tyrosinase activity both in vivo and in vitro has a potential diagnostic and therapeutic application. Small-molecule fluorescent probes have become a powerful device over the traditional biochemical method for the detection and imaging of enzymatic activities in biological systems by virtue of their superior sensitivity, nondestructive fast analysis, spatiotemporal resolution and real-time detection abilities. Moreover, due to their structural tunability, several small-molecule fluorescent probes have been developed to meet various aspects such as enhancing sensitivity, selectivity, cell permeability, real-time monitoring and easy imaging in biological systems. This review article sums up the recent progress of small-molecules fluorescent probes for tyrosinase activity, including their synthesis strategies, mechanistic paths and potential applications based on reports mainly in the past five years. The rapid advancement in this field suggests that fluorescence detection and imaging is a promising technology and widen up new horizons for early diagnosis of melanoma.

Keywords: Tyrosinase, Melanoma, Fluorescent, Probe, Biomarker, Cell

***Corresponding Author**

Krishanu Sarkar , Assistant Professor, Department of Chemistry, Netaji Mahavidyalaya, Arambagh, Hooghly, 712601, West Bengal, India

Recieved On 23 May 2020
Revised On 26 June 2020
Accepted On 14 July 2020
Published On 10 April 2021

Funding This Review did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

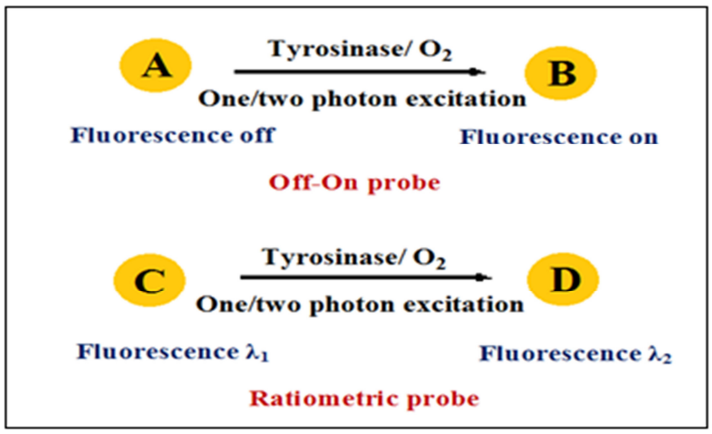
Citation Krishanu Sarkar , Recent Advancement in Fluorescent Probes for Sensing and Imaging Tyrosinase Activity in Living Cells.(2021).Int. J. Life Sci. Pharma Res.11(2), L1-17 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.2.L1-17

1. INTRODUCTION

Tyrosinase, a copper-containing mono-oxygenase, is widely distributed in plants, animals and microorganism[1]. It catalyzes the hydroxylation reaction of phenol derivatives to the corresponding oxidative catechol derivatives followed by further oxidation to the corresponding ortho-quinone products[2] which can trigger melanogenesis in melanocytes[3]. Tyrosinase also plays an important role for biosynthesis of melanin pigments, which is the most vital determinant of the eye, hair, skin color[4], protects the melanocytes DNA from ultraviolet radiation and removes reactive oxygen species[5,6]. This tyrosinase is also regarded as a biomarker of melanoma because its overexpression plays an important role in the development of melanoma[7]. Moreover, dysfunction of tyrosinase causes numerous diseases. For example, people with insufficient melanin are prone to albinism or vitiligo, and thus are very sensitive to ultraviolet exposure with a high risk of skin cancer[8,9]. Conversely, the excessive expression of tyrosinase is probably associated with malignant melanoma[10]. Additionally advanced research showed that tyrosinase might contribute to the dopamine neurotoxicity and neurodegeneration which are responsible for Parkinson's disease[11]and Schizophrenia[12]. Therefore, development of a highly precise and sensitive probe for this biochemical marker is very much desirable for both understanding its role in biological and pathological processes and its diagnostic approaches in biomedical research. Different traditional methods such as electrochemical assay, liquid chromatography, gas chromatography, and colorimetric techniques were employed for the diagnosis of tyrosinase activity[13-17]. However, sophisticated instrumentation, time-consuming process, and skilled man power limit their applications. Conversely, fluorescence techniques have gained significant interest because of its high sensitivity, easy accessibility, significant spatiotemporal resolution and noninvasive nature in imaging and detection of biological analytes[18-21]. Based on the sensing mechanism fluorescent probes are of two types such as 'turn-on' type sensor (fluorescence enhancement) and 'turn-off' type sensor (fluorescence quenching). 'Turn-off' type fluorescent sensors have not gained a lot of attention because of many limitations such as poor signal-to-background ratio, susceptible to external parameters etc. But sensors based on 'turn-on' are very popular to researchers because of its smart approach to design various "turn-on" fluorescent probes by incorporating recognizing groups into fluorophore scaffolds[22,23]. Again the fluorescence response mechanisms exhibited by the organic probes of tyrosinase assays mainly operated in two paths, the first one is oxidation-cleavage path[24-26] where tyrosinase oxidize hydroxyphenyl substrate to ortho-quinone and eliminate fluorophore from the substrate by enhancing fluorescence intensity and in the other tyrosinase triggers the fluorescence intensity of the probe by inhibiting photo induced electron transfer process[27,28]. On the other hand, turn on/turn off type fluorescence probes often suffer in auto fluorescence, small stoke shifts and low penetration to living cells[29,30] along with some external interferences such as concentration, temperature, excitations wavelengths and resulting false reporting. But ratiometric fluorescent probes[31-33] overcomes such problems because such sensors give responses to the analytes at two different wavelengths, thus increasing the accuracy[34,35] of the result by eliminating external factors. The ratiometric signals produced by these probes are mostly based on the intramolecular charge transfer (ICT) and fluorescence resonance energy transfer (FRET) process. Again, majority of the probes used 4-hydroxyphenyl moiety or its derivative as a recognition unit for tyrosinase assay but 4-substituted unit display interference in the presence reactive oxygen species such as HOCl, H_2O_2, and many other free radicals[36,37]. By introducing a meta hydroxy phenyl unit into the fluorescent probe as a recognition unit [38,39] such interferences were subsided. This review outlines the recent progress in the development of single molecule fluorescent probes for the sensing and imaging of tyrosinase activity in vitro and/or in vivo. It is worth mentioning that other fluorescent probes based on quantum dots[40], noble-metal nanoclusters[41], polymeric nanoparticles[42], are not covered here. This review covers the various design strategies, sensing mechanism and biological application of the fluorescent probes reported by the renowned researchers and also the existing challenges in achieving high selectivity and sensitivity towards tyrosinase activity.

2. *DESIGN STRATEGIES OF FLUORESCENT PROBES*

Among the various fluorescent probes, 'off-on' probes and ratiometric probes are highly desired because of some characteristic features such as low background signal, high spatiotemporal resolution etc. On the other hand two photon absorption active fluorescent probes are especially important in the biological field because two photon excitation facilitates deep penetration, less light scattering and minimized auto-fluorescence background. Here mainly two kinds of fluorescent probes for the assay of tyrosinase activity are discussed: off-on probes and ratiometric probes (Scheme 1)[43]



Scheme 1. Design strategies of fluorescent probes for tyrosinase activity assay. Adapted with permission from ref. 43. Copyright © 2020, Elsevier.

3. *DIFFERENT RECOGNITION UNITS FOR TYROSINASE ACTIVITY*

In general, the active sites of tyrosinase[43a] (Figure 1) contain two copper atoms with six histidine molecules. Again for the successful assay of tyrosinase activity fluorescent probes must have two parts, one part for generation of fluorescence and the other part is for recognition of tyrosinase. There are mainly two types of tyrosinase recognition unit reported, phenols substituted at the 4-position, and 3-position. 4-position substituted phenol often suffers from interference by reactive oxygen species but the 3-position substituted phenol can overcome this interference problem. Both types of probes could be successfully used in tracing tyrosinase activity in living cells.

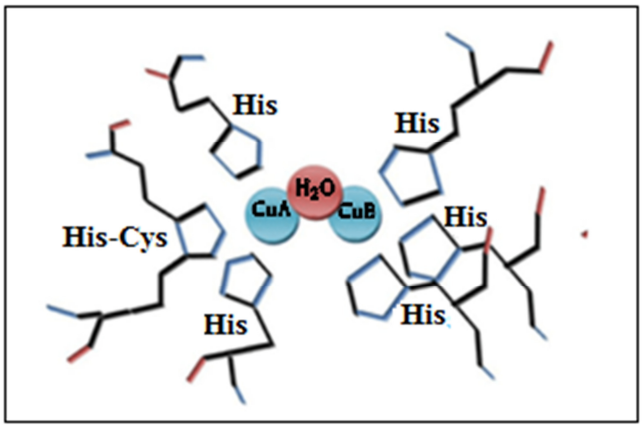
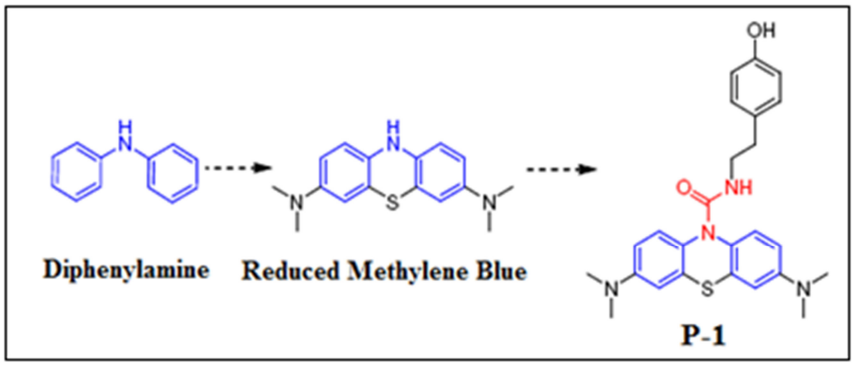


Figure 1. General crystal structure of tyrosinase. Adapted from ref. 43a.

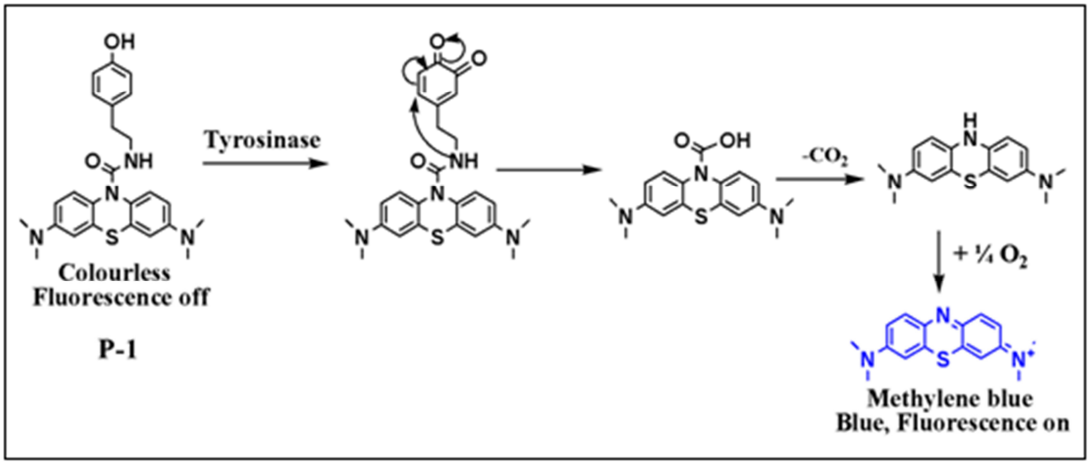
3.1 *4-Hydroxyphenyl based fluorescent probes*

A highly sensitive near-infrared probe (P-1) for monitoring tyrosinase activity was reported by Li and his group[44]. Methylene blue by virtue of its imaging property[45], was used in its reduced form to synthesize a tyrosinase probe, P-1, with a diphenylamine-type urea bond (Scheme 2a).Molecular docking experiment proved that the probe can insert into the catalytic pocket of tyrosinase without causing any steric hindrance.



Scheme2a. Design of the fluorescent probe, P-1, for tyrosinase activity. Adapted with permission from ref. 44. Copyright © 2018, American Chemical Society.

The probe solution in the potassium phosphate buffer does not show any fluorescence emission. But in presence of tyrosinase solution (5U mL^{-1}) the fluorescence intensity of P-1 increased greater than 100-fold within 20 min with the colour changed to light blue. The proposed reaction mechanism for the release of methylene blue from P-1 in the presence of tyrosinase is shown in Scheme 2b. P-1 probe first oxidized to the corresponding ortho-quinone, then undergoes a rapid intramolecular cyclization process and triggers the hydrolysis of the urea bond resulting in the release of methylene blue through amide intermediate via oxidation reaction[46].



Scheme 2b. The proposed reaction mechanism of the probe P-1. Adapted with permission from ref. 44. Copyright © 2018, American Chemical Society.

In a cell study experiment, B16F10 melanoma cells (mouse) showed a bright fluorescent signal after incubation with P-1 and the fluorescence intensity was almost 7.5-fold higher than the control (Figure 1a). To prove that the observed fluorescence comes from methylene blue after tyrosinase catalysis, B16F10 cells were firstly treated with kojic acid (a commercial tyrosinase inhibitor) and then treated with P-1 and kojic acid simultaneously. As expected, the fluorescence intensity of B16F10 cells was reduced. Again when HeLa cells which express low levels of tyrosinase, were treated with P-1, the fluorescence intensity was significantly lower than that of B16F10 cells.

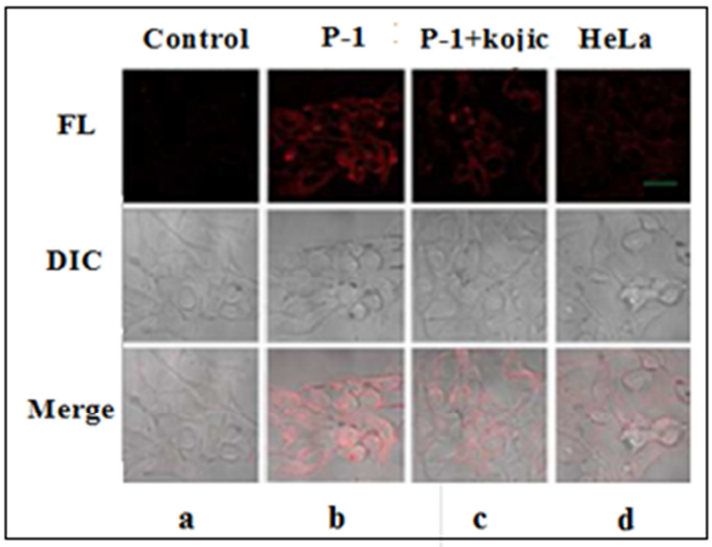
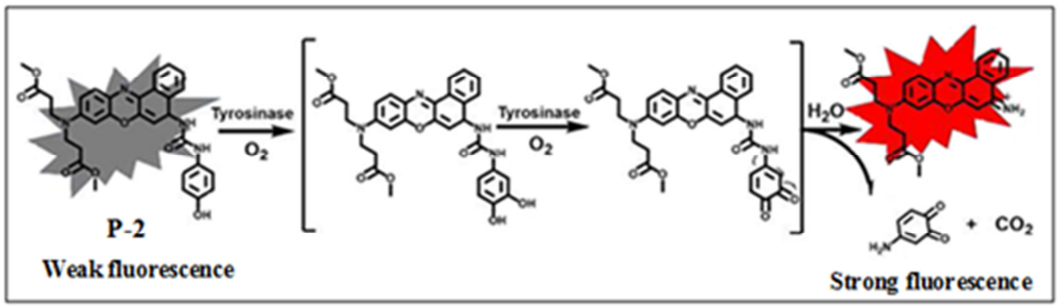


Figure 1a. Confocal (FL) and differential interference contrast (DIC) microscopy images of B16F10 and HeLa cells (a) B16F10 control cells (untreated). (b) B16F10 cells treated with P-1 (c) B16F10 cells treated with P-1 and kojic acid (d) HeLa cells treated with P-1. Adapted with permission from ref. 44. Copyright © 2018, American Chemical Society.

Later Zhan et al.[47] developed a novel fluorescent probe, P-2, for monitoring tyrosinase activity and early detection of melanoma cells. In this probe hydroxyphenylurea group was attached as substrate part for recognition to tyrosinase and a fluorescent dye phenoxazine derivative as signal reporter. The probe was synthesized by linking the tyrosinase recognition element ((4- hydroxyphenyl)urea) with the phenoxazine derivative by forming the urea linkage (Scheme 3).



Scheme 3. Schematic diagram for detection of tyrosinase by the probe P-2. Adapted with permission from ref. 47. Copyright © 2018, American Chemical Society.

The probe, P-2, shows a weak absorption peak around 510 nm and upon interaction of tyrosinase, the peak appeared at 580 nm. Accordingly, P-2 alone showed almost no fluorescent emission whereas 7-fold enhanced intensity at 660 nm upon treatment with tyrosinase. The probable mechanism attributed to the breaking of the carbamide bond in presence of tyrosinase and producea free amine group in the dye resulting regaining of its fluorescence and thus achieving the fluorescent detection of the tyrosinaseactivity. Moreover, the probe is able to sensitively and selectively detect the endogenous tyrosinase level in live cells and in zebrafish in PBS buffer medium (Figure 2). More significantly, this probe is capable of real-time early diagnosis of melanoma in the mouse model.

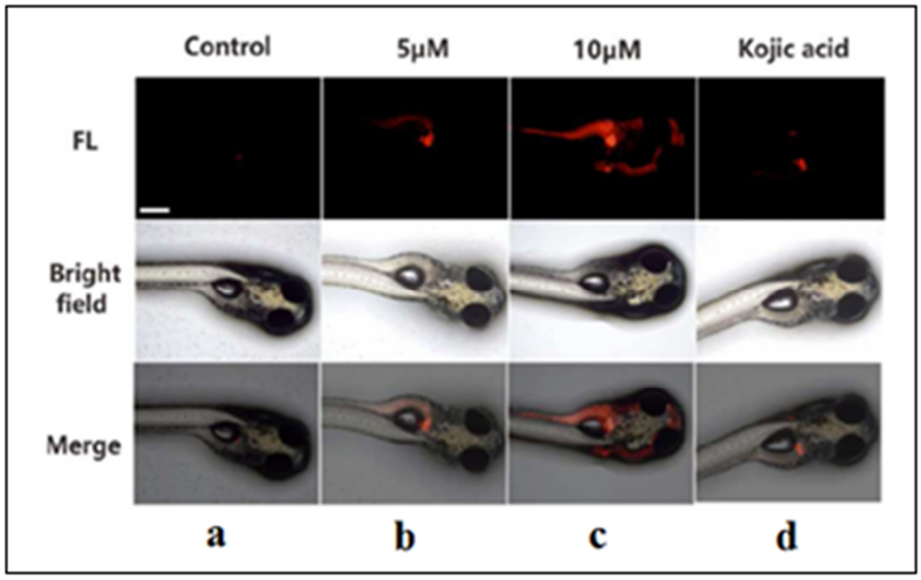
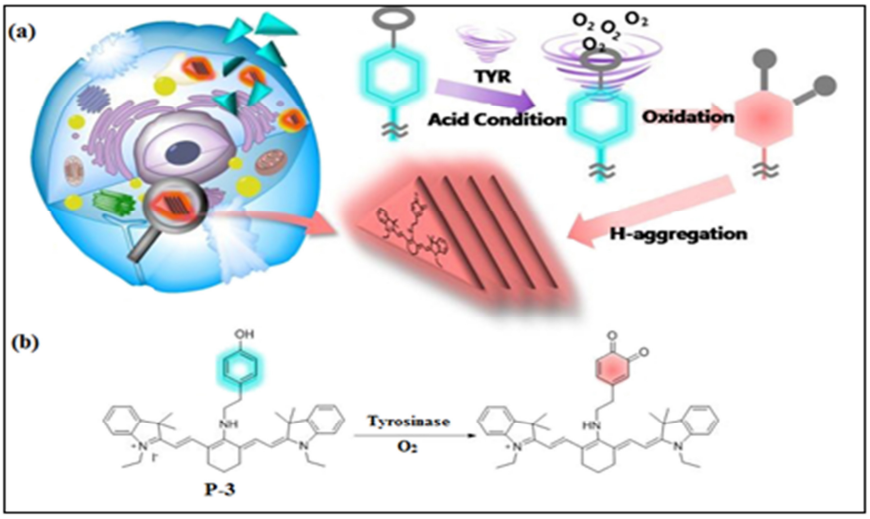


Figure 2. Images for live cell study of 3-day-old zebrafish: (a) untreated zebrafish (the control), zebrafish incubated with 5 μMP-2 (b), zebrafish incubated with 10 μM P-2 (c) and zebrafish treated with 400 μM kojic acid and then incubated with 5 μM P-2 (d) Scale bar: 200μm. Adapted with permission from ref. 47. Copyright © 2018, American Chemical Society.

For the first time a novel cyanine-based ratiometric fluorescent probe (P-3) was reported by Zhang and his co-worker[48] for tyrosinase (TYR) activity based on aggregation and near infrared absorbance nature of cyanine dye. In presence of oxygen, when TYR was treated with P-3, the phenolic hydroxyl group was oxidized into o-benzoquinone, and the resulting cyanine products undergo H-aggregation to induce fluorescence enhancement (Scheme 4). This probe P-3 exhibits a maximum absorption at 630 nm and emits in the NIR region, centered at ca. 760 nm. But during tyrosinase activity, the absorption peak of P-3 shifted to 516 nm and fluorescence emission at 560 nm because of the cyanine aggregation. The ratiometric fluorescent probe, P-3 showed excellent signal-to-noise ratio, leading to a high sensitivity for TYR activity with a limit of detection 0.02 U mL^{-1}.



Scheme 4. (a) Proposed sensing mechanism for TYR recognition by the probe P-3 (b) Molecular structures of probe P-3 and enzymatic product. Adapted with permission from ref. 48. Copyright © 2019, Royal Society Chemistry.

The real-time imaging of TYR activity in B16 cells was thoroughly studied. After incubation with 3 μM of P-3, B16 cellular imaging was recorded in 1 h interval as shown in Figure 3. During the investigation, a gradual increase in emission intensity in the red channel was noticed along with a remarkable drop in NIR emissive window. These fluorescence studies established that the probe P-3 possessed superior membrane permeability and biocompatibility and suitable for monitoring TYR level in living cells.

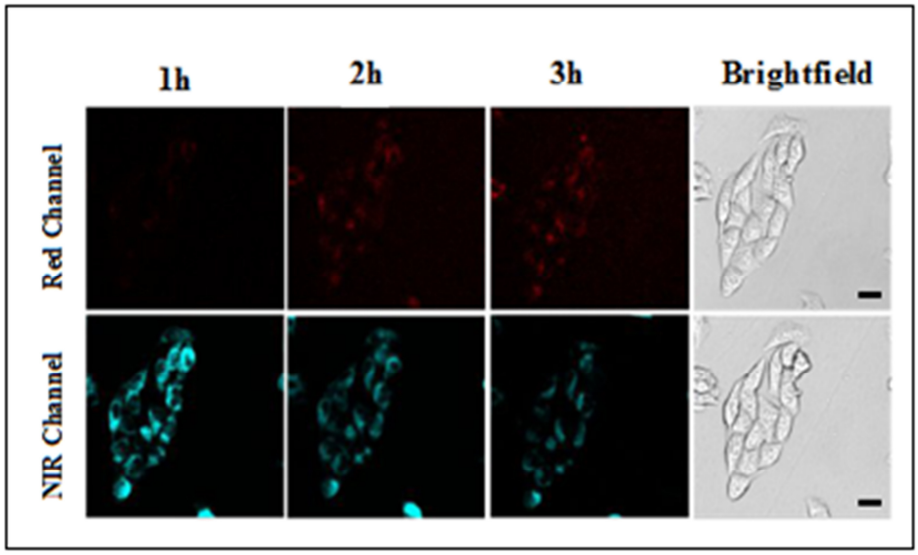
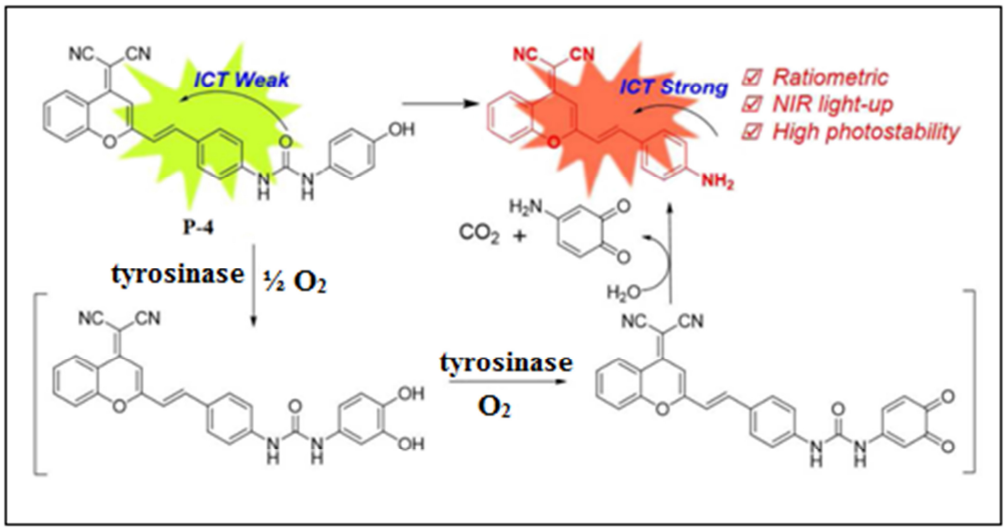


Figure 3. Time dependence real-time imaging of B16 cells incubated with the probe P-3 (3 μM). Adapted with permission from ref. 48. Copyright © 2019, Royal Society Chemistry.

In the same year Li et al.[49] developed a TYR activatable near infrared fluorescent probe P-4, where dicyanomethylene-4H-pyran (DCM) moiety was incorporated as the NIR fluorescence reporter and phenol group as the triggered unit. The interaction of P-4with endogenous TYR can initiate a significant fluorescence red shift from 548 nm to 660 nm and thus allowing for ratiometric and light-up NIR response probe for monitoring TYR activity endogenously. The probable sensing mechanism involves that first P-4 probe exhibits a typical ICT process resulting quenching of NIR fluorescence of DCM-NH_2. Then after treatment with TYR, DCM-NH_2 is specifically triggered and released from the probe P-4, restoring its electron donor ability and then exhibited a remarkable ratiometric fluorescence signal (Scheme 5). In fact, the probe P-4 has the initial emission at 548 nm, but after incubation with TYR, a new peak at about 660 nm was observed and simultaneous weakening at 548 nm. The detection limit of the probe P-4 was found to be 0.6 U/mL by applying standard 3σ/k method.



Scheme 5. Proposed sensing mechanism for TYR enzymatic activation of P-4. Adapted with permission from ref. 49. Copyright © 2018, Elsevier.

Cell study experiment of B16 cells showed (Figure 4) that almost no background fluorescence (image A-D in Figure 4; control) for the cell itself. After incubation with 5 μM P-4 probe for 3 h, B16 cells exhibited a fluorescence quenching in green channel but fluorescence enhancement in red channel (Figure 4F, G) in a ratiometric fashion. Figure 4D, H and L represents the corresponding ratiometric images constructed from red and green channel. This established that P-4 has enough cell-permeability to react with TYR in B16 cells. They also performed an inhibitor experiment to know the origin of NIR fluorescence. When the cells treated with of kojic acid (inhibitor), the strong NIR fluorescence signal became appreciably weaker (Figure 4K). Therefore in vitro experiments of B16 cells by P-4 probe showed that this probe could be used for monitoring tyrosinase activity inside the living cell. Moreover, P-4 exhibits excellent photostability and low cytotoxicity which also trigger the probe for elucidating the roles of TYR in biological systems.

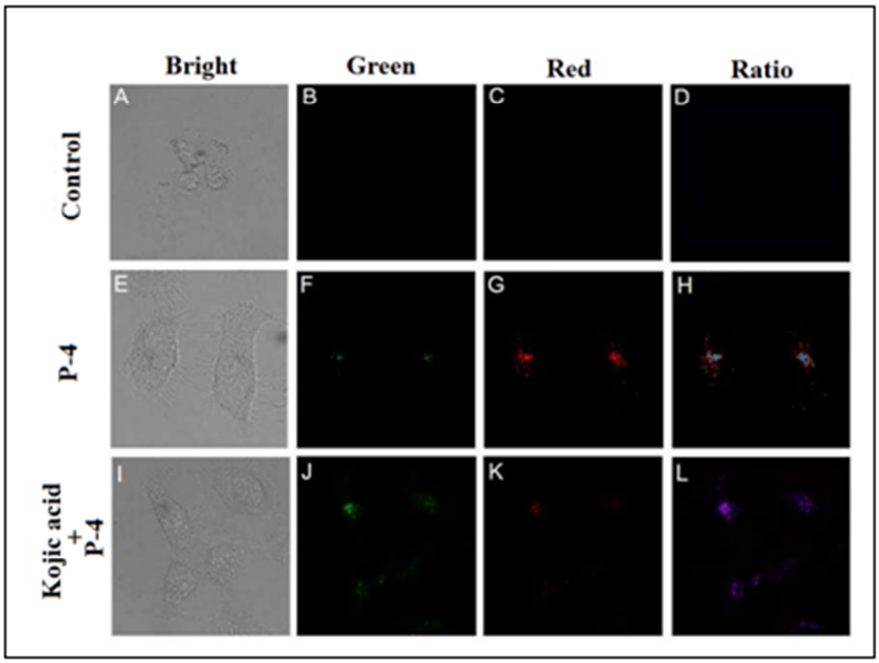
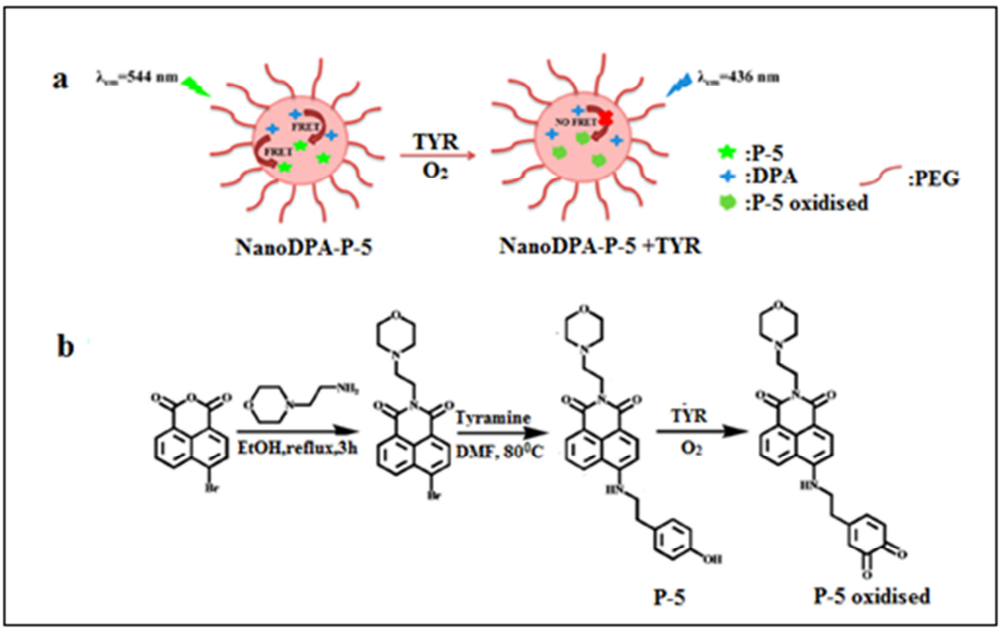


Figure 4. Confocal laser scanning microscopy images of B16 cells: cells only (control, A-D), cells incubated with probe P-4 (5μM) (E-H), cells pretreated with kojic acid (200 μM) then incubated with probe P-4 (5 μM) (I-L). The green channel was collected from 500 to 560 nm, the red channel was collected from 630 to 700 nm, and ratiometric images constructed from red and green channels; λex = 450 nm. Scale bar, 10 μm. Adapted with permission from ref. 49. Copyright © 2018, Elsevier.

In the meantime Wang and his group[50] reported a Förster resonance energy transfer (FRET) based[26] novel self-assembling ratiometric fluorescent micelle nanoprobe, P-5 (NanoDPA-NMP-tyr) for tyrosinase (TYR) assay in B16 cells.

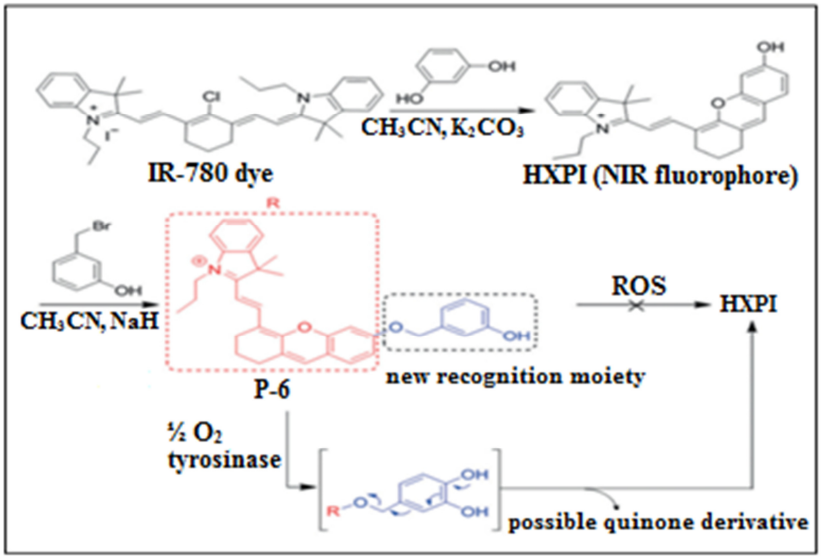


Scheme 6. (a) Schematic diagram of the FRET mechanism (b) Synthesis of P-5 and its response to TYR. Adapted with permission from ref. 50. Copyright © 2019, American Chemical Society.

This probe P-5, which was composed by a naphthalimide derivative and a recognition group tyramine, and the dye 9,10- diphenylanthracene (DPA) were encapsulated with amphiphilic copolymer 1,2-dimyristoyl-sn-glycero-3-phospho-ethanolamine-N-(methoxy(polyethylene glycol)-2000) (mPEG-DSPE). Because of the overlap of the emission band of the dye DPA (λem= 445 nm) with the absorption band of P-5 (λabs=450 nm), the FRET mechanism operates. In NanoDPA-P-5, P-5 is serves as energy acceptor and DPA is used as a donor and FRET operates (Scheme 6a).In presence of TYR and O_2,the monophenol derivative of the tyrosine was oxidized by TYR to produce corresponding quinine product (Scheme 6b).As a result absorption peak of the P-5 was blue shifted by about 100 nm consequently no overlap of the fluorescence emission spectrum of DPA with the absorption spectrum of P-5 take place and so FRET was inhibited blocked and change in fluorescence from P-5 green to DPA blue occurs thus achieving a very fast (response time of 7.5 min) ratiometric response to TYR. Again, because of good water solubility and low cytotoxicity, this NanoDPA-P-5 fluorescent nanoprobes find application clinical diagnosis and in real-time fluorescence imaging of TYR in living cells with excellent results. The NanoDPA-P-5 probe is highly selective and sensitive toward TYR with a rapid fluorescence response, and the detection limit of TYR is 0.057 U/mL.

3.2 *3-Hydroxyphenyl based fluorescent probes*

4-hydroxyphenyl-based probes for the recognition of tyrosinase activity have many advantages such as easy availability of starting material, simple synthesis steps, and cheap reagents, but they often suffer from poor selectivity as both tyrosinase enzyme and reactive oxygen species such as HOCl, H_2O_2, whose concentration is greater than tyrosinase, oxidize recognition groups to corresponding quinones[51], and as a result produce wrong experimental results. But with the use of 3-hydroxyphenyl based probes such interference phenomena can be overcome. Wu and his co-workers[52] developed a near-infrared (NIR) fluorescence probe, P-6, based on a hemicyanine skeleton with a 3-hydroxybenzyloxy moiety as recognition unit for tyrosinase activity. 3-hydroxy unit in 3-hydroxybenzyloxy moiety accelerates the hydroxylation at the vacant 4-position by tyrosinase only but not by reactive oxygen species (ROS) and thereby the resulting hydroxylation unit would be spontaneously removed by the subsequent 1,6-rearrangement-elimination reaction (Scheme 7). The probable reason may be due to direct coordination of active copper of the tyrosinase active site with phenolic substrates. Moreover, the selectivity over tyrosinase of the probe P-6 was further confirmed by simultaneous treatment with commonly co-existing biological substances such as inorganic salts, glucose, vitamin C, vitamin B6, glycine, glutamic acid, cysteine, glutathione, creatinine, urea, and some enzymes etc.



Scheme 7. Synthesis of P-6 and the proposed reaction mechanism with tyrosinase and ROS. Adapted with permission from ref. 52. Copyright © 2016, AngewandteChemie International Edition.

In biological cell study, murine melanoma B16 cells and 3-day-old living zebrafish were chosen as a model because these types of cells show overexpressed tyrosinase. As presented in the Figure 5A, B16 cells itself show an extremely low fluorescence (image a), which benefits from the NIR excitation wavelength of the probe. However, the B16 cells treated with probe P-6, exhibit strong fluorescence (image b), suggesting a good cell-permeability for P-6 and its possible reaction with tyrosinase in the cells. To establish that the fluorescence enhancement was due to the presence of tyrosinase, kojic acid (inhibitor of tyrosinase) was used to pretreat the cells, and the pretreated cells generated a notable lower fluorescence (image c). This proves that the fluorescence enhancement in B16 cells was due to the presence of endogenous tyrosinase. Similarly in the living zebrafish experiment, 3-day-old zebrafish display nearly no background fluorescence (Figure 5B, image a), but after incubation with the probe P-6, zebrafish produce strong fluorescence (image b). This indicates that the probe P-6 is cell-permeable and zebrafish contain a noticeable tyrosinase level but the images support the non uniform distribution of tyrosinase. Similarly the fluorescence enhancement comes from the presence of tyrosinase was proved by treating with kojic acid (image c and d).

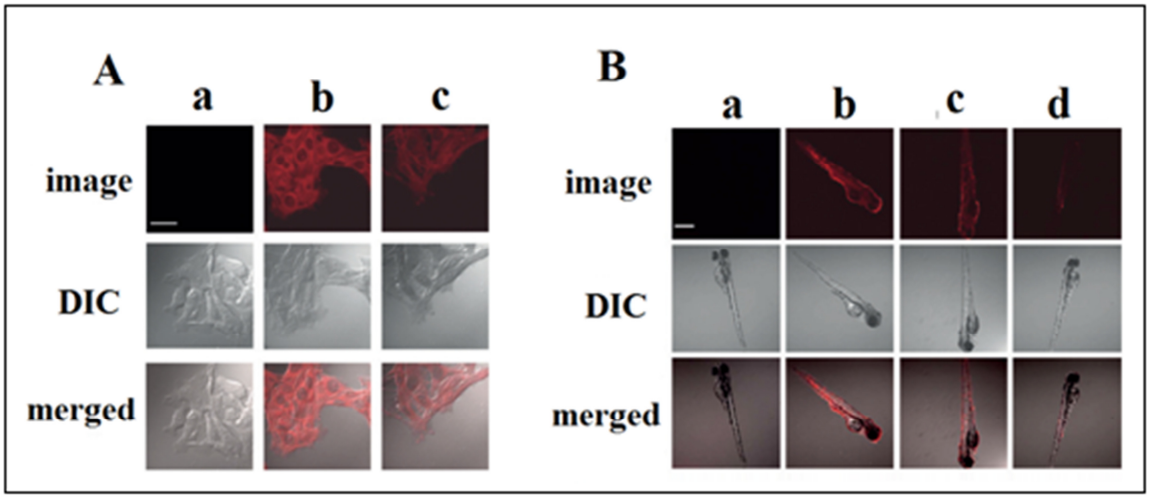
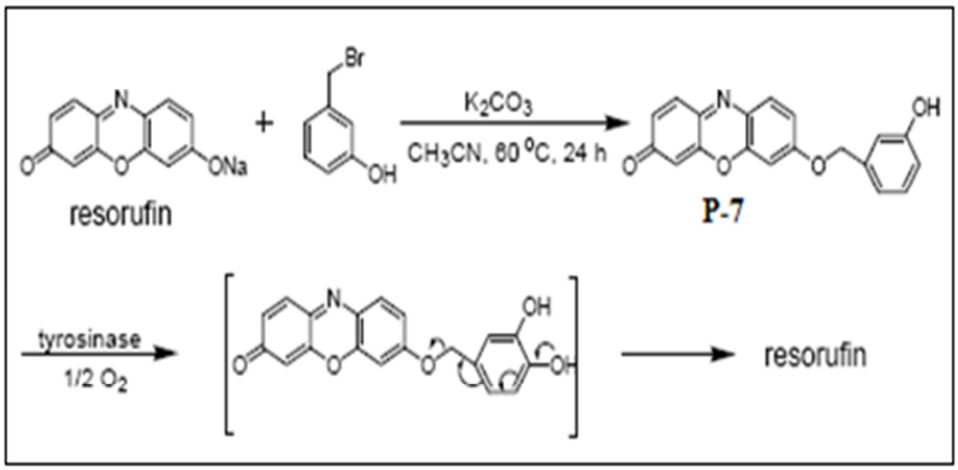


Figure 5. A) Confocal fluorescence images of B16 cells: a) cells only (control) b) cells incubated with P-6 (5 mm) for 3 h c) cells pretreated with kojic acid (200 mm) for 2 h and then incubated with P-6 (5 mm) for 3 h. The differential interference contrast (DIC) and merged images of the corresponding samples are shown in the middle and bottom rows, respectively. Scale bar=30 mm B) Fluorescence images of 3-day-old zebrafish: a) zebrafish only, b) zebrafish incubated with P-6 (5 mm) for 3 h, zebrafish pretreated with c) 200 mm and d) 500 mm kojic acid for 2 h and then incubated with P-6 (5 mm) for 3 h. The top, middle, and bottom rows represent the fluorescence, DIC, and merged images of zebrafish, respectively. Scale bar=500 mm. Adapted with permission from ref. 52. Copyright © 2016, AngewandteChemie International Edition.

Later on the same group[38] developed a novel resorufin-based fluorescent off-on probe, P-7, using m-hydroxyphenyl unit as the recognition moiety of tyrosinase (Scheme 8) for the trace detection of tyrosinase activity in biological samples. The probe P-7 was synthesized in a single step reaction just by coupling resorufin salt with 3-(bromomethyl)phenol. Here

the dye resorufin was chosen as a fluorescent chromophore because of its alkylation at 7-hydroxy group produces a nearly complete fluorescence quenched[53] product which achieves an extremely low background signal and thus make it a sensitive assay. Again, the presence of 3-hydroxyphenyl group in probe P-7 triggers the hydroxylation at the adjacent vacant 4-position by tyrosinase selectively and the formed hydroxylation unit spontaneously detached via subsequent 1,6-rearrangement-elimination reaction and thus creating a fluorescence off-on response. The probe P-7 showed high sensitivity and selectivity for tyrosinase activity over other reactive oxygen species justifying its potential use in recognizing endogenous tyrosinase activity and imaging in different living cells.



Scheme 8. Synthesis of P-7 and its sensing mechanism. Adapted with permission from ref. 38. Copyright © 2016, AngewandteChemie International Edition.

In detecting endogenous tyrosinase activity in living cells, murine melanoma B16 cells were taken because of its overexpressed tyrosinase content. It was clear from Figure 6 that B16 cells itself show almost no background fluorescence (image a in Figure 7, control), but the cells treated with P-7displays strong fluorescence (image b), justifying good cell-permeability and sufficient interaction of P-7 with tyrosinase inside the cells. Again the inhibitor experiment with kojic acid proved the generation of fluorescence responsible for the interaction of tyrosinase with P-7. The Probe P-7 displays high sensitivity and selectivity towards tyrosinase with a detection limit of 0.04 U/mL.

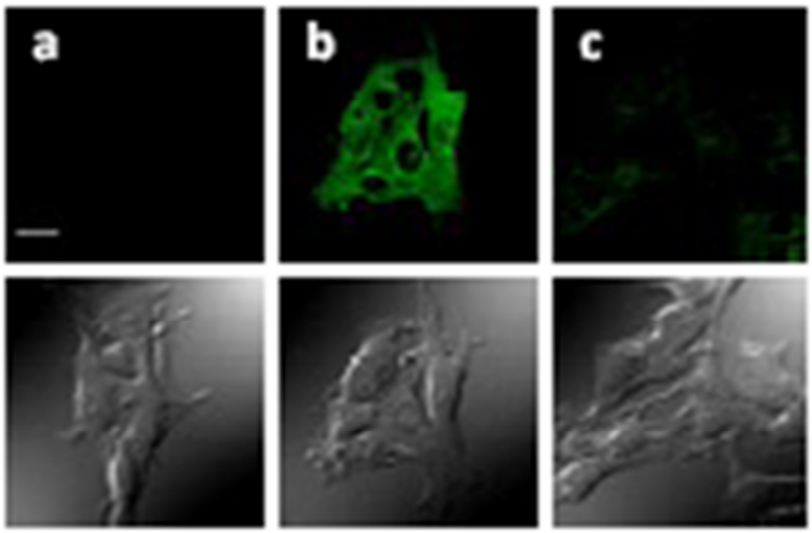
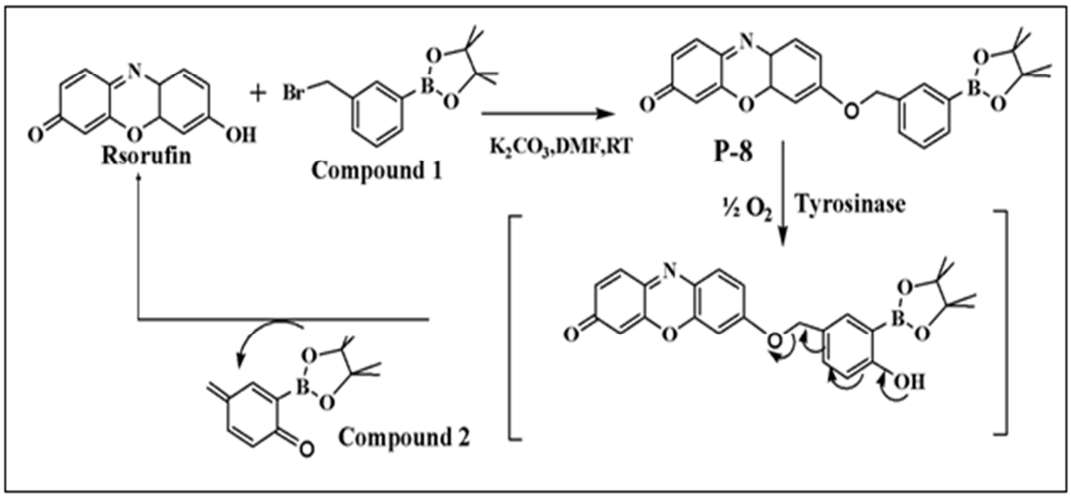


Figure 6. Fluorescence images of B16 cells. (a) B16 cells only (control). (b) The B16 cells were treated with probe P-7 (5 μM) for 3 h. (c) The B16 cells were pretreated with kojic acid (200μM) for 1.5 h, and then incubated with probe P-7 (5 μM) for 3 h. The second row displays the differential interference contrast (DIC) images. Scale bar, 20 μm. Adapted with permission from ref. 52. Copyright © 2016, AngewandteChemie International Edition.

A novel fluorescent tyrosinase probe, P-8, was reported by Li and his team[54]. They introduced resorufin dye as the fluorophore unit and m-tolylboronic acid pinacol ester as the receptor unit for tyrosinase (Scheme 9).



Scheme 9. Synthesis route of the probe P-8 and tyrosinase-catalyzed reaction. Adapted with permission from ref. 54. Copyright © 2017, Royal Society of Chemistry.

Sensing mechanism operated via hydroxylation of adjacent position of the phenyl boronic acid pinacol ester in probe P-8 by tyrosinase, and simultaneous release of resorufin unit via 1,6-rearrangement-elimination reaction. The function of tyrosinase is to modulate the hydroxylation at the adjacent position of phenyl boronic acid pinacol ester and also form coordination bond with phenyl boronic acid pinacol ester via two copper sites. This probe P-8, shows an absorption band at 475 nm and after interaction with tyrosinase, a new absorption band appears at 570 nm. Similarly, the probe P-8 shows almost no emission due to the alkylation of the 7-hydroxy group in the resorufin unit but the strong fluorescence emission appears at 583 nm after interaction with tyrosinase. Again the fluorophore resorufin displays absorption spectra at 570 nm with corresponding emission at 583 nm. This establishes that the generation of fluorescence emission in probe P-8 originates because of the presence of a resorufin dye unit in the probe. This probe quantifies tyrosinase activity in the range from 1 to 100 U mL^{-1} with an acceptable detection limit of 0.5 U mL^{-1}. Then fluorescence imaging of tyrosinase in living cells was conducted with melanoma cell line, B16 cells. As shown in the image of Figure 7, B16 cells display almost no fluorescence, whereas the images b exhibit strong fluorescence when incubated with the probe, and when B16 cells treated with kojic acid and the probe the image c appears with weak fluorescence, establishing that the probe P-8 can be applied to monitor intracellular tyrosinase activity. Again when the probe P-8 is treated with HepG2 cells (non-melanoma cells), exhibit almost no fluorescence, image d, because of weak expression of tyrosinase.

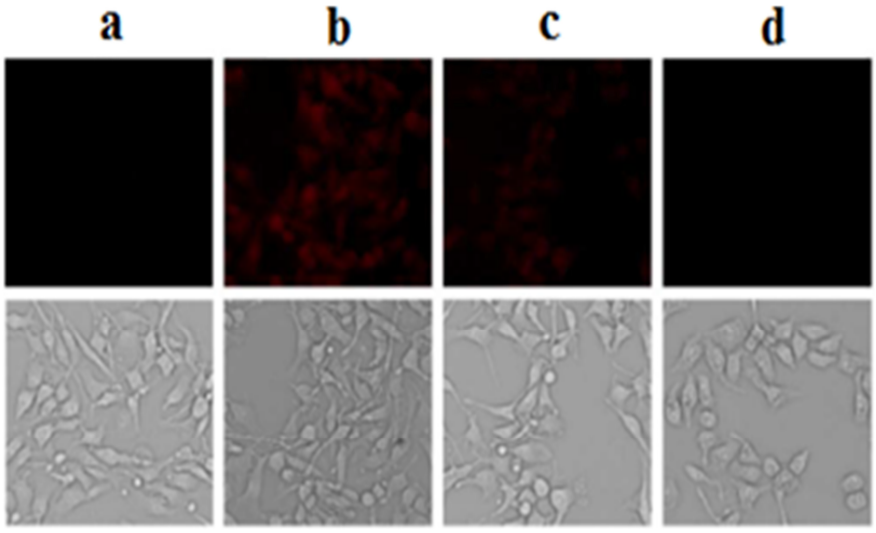
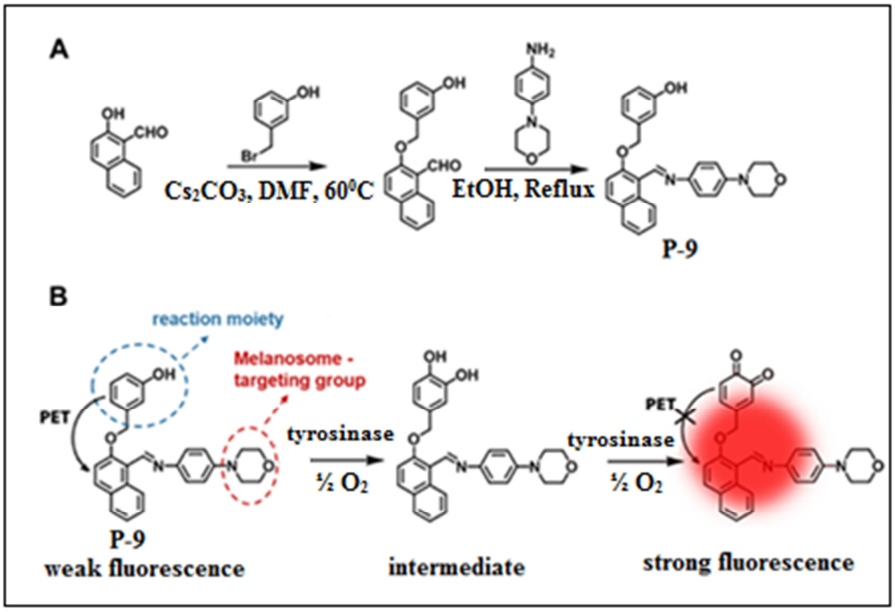


Figure 7. Fluorescence images. (a) B16 cells (b) B16 cells incubated with probe P-8 (2.5 μM) for 5 h(c) B16 cells pretreated with kojic acid (200 μM) for 2 h and then incubated with P-8 (2.5 μM) for 5 h, (d) HepG2 cells treated with probe P-8 (2.5 μM) for 5 h. The second row displays the corresponding bright field images. Adapted with permission from ref. 54. Copyright © 2017, Royal Society of Chemistry.

A new melanosome-targeting near-infrared (NIR) fluorescent probe (P-9) was synthesized by Peng et al.[39] by introducing m-hydroxybenzyl unit for recognizing TYR specifically and the morpholine moiety that facilitates easy interaction with melanosome into salicylaldazine skeleton for in situ detection of intracellular TYR. The probe P-9 itself shows low fluorescence emission due to the m-hydroxybenzyl moiety that operates a photo-induced electron transfer (PET) effect towards the naphthalene moiety of the probe but after interaction with tyrosinase, the PET process is inhibited by

the oxidation of the phenol unit to the corresponding benzoquinone resulting in an intense "turn-on" fluorescent response (Scheme 10). Again, P-9 after interacting with tyrosinase, shows a good NIR characteristic having absorption at 480 nm and subsequent enhancement of fluorescent emission intensity (48 fold) at 675 nm with a large Stokes shift of 195 nm which effectively reduces background fluorescence caused by excitation light and scattered light. This new probe is very much suitable for the detection of intracellular endogenous TYR activity with a detection limit of 0.5 U mL^{-1}.



Scheme 10. (A) Synthesis of probes P-9 (B) and proposed fluorescence turn-on mechanism for TYR activity. Adapted with permission from ref. 39. Copyright © 2018, American Chemical Society.

This probe P-9 has been successfully applied in imaging and in situ quantification of the intracellular TYR activity. To exploit the excellent sensing properties of the probe in living cells, first its cytotoxicity experiment was done by MTT assays which show good viability (90 % above) towards B16 cells. Here in the biological study, the probe was applied in detecting the endogenous TYR activity in living cells, such as melanoma cells B16, cancer cells (HepG2, A549 and HeLa) and normal cells (HPF and HSF). The deviation of fluorescence intensity indicated the different expression levels of TYR in different types of cells (Figure 8). It is clear from the cell study that the melanoma cells exhibit highest fluorescence signals compared to other cancer cells and normal cells, probably because of over-expression of tyrosinase activity in B16 cells over other cells.

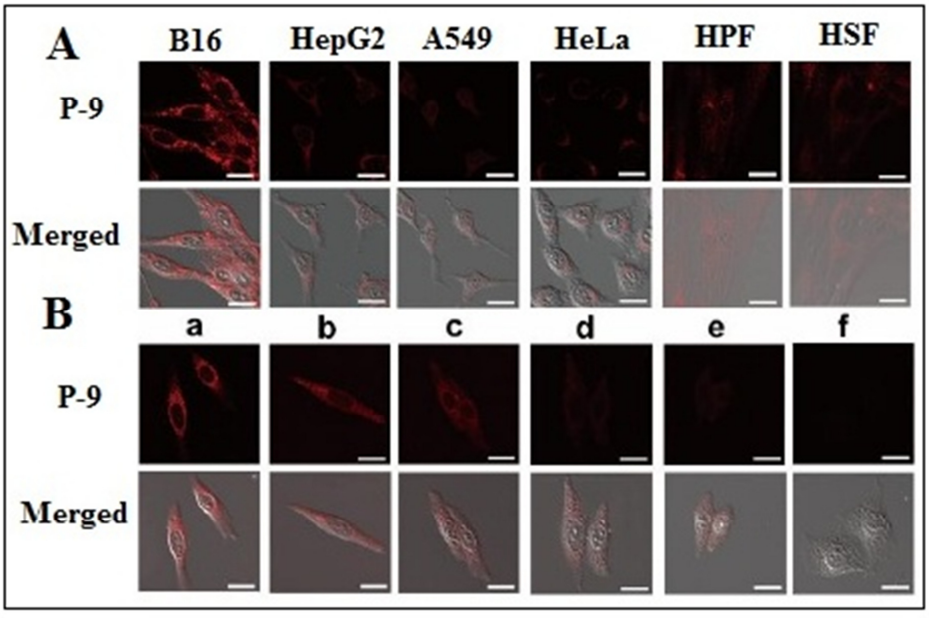
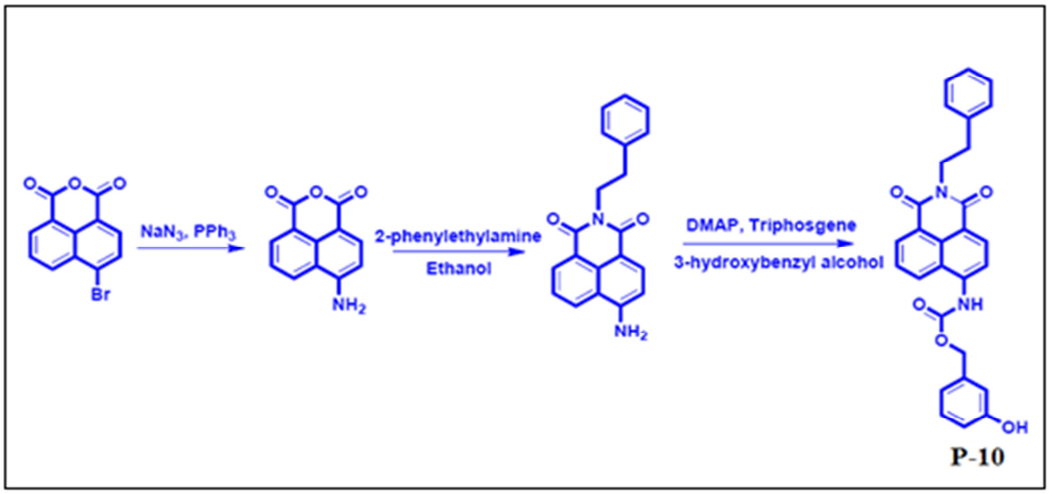


Figure 8. (A) Cellular images of TYR in B16, HepG2, A549, HeLa, HPF, HSF cells after incubation with 10 μM P-9 for 12 h respectively. (B) Variation of cellular images of TYR in B16 cells pretreated with 0, 20, 50, 100 and 200 μM kojic acid for 3 h

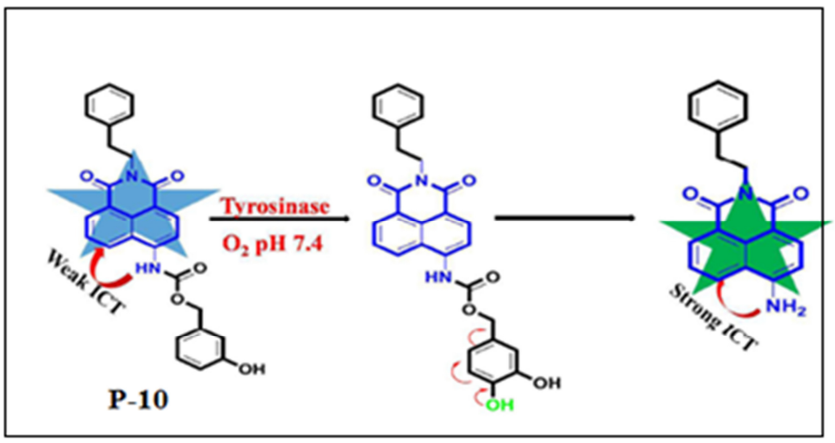
(from a to e) and then incubated with 10 μM P-9 for 12 h, and confocal images of B16 cells only (f). Adapted with permission from ref. 39. Copyright © 2018, American Chemical Society.

Later Sidhu and his group[33]developed a reliable ratiometric fluorescence sensor probe, P-10, for the selective detection of tyrosinase having a m-hydroxyphenyl unit for tyrosinase response in conjugation with 4-aminonaphthalimide unit which act as fluorophore. The probe P-10, was synthesized by a single step coupling reaction between 4-aminonaphthalimide and 3-hydroxybenzyl alcohol as shown in Scheme 11.



Scheme 11. Synthesis steps of fluorescent probe P-10. Adapted with permission from ref. 33. Copyright © 2018, Royal Society of Chemistry.

4-aminonaphthalimide shows high photostability and good cell permeability. Again the 4-amino group extensively engaged in intramolecular charge transfer to imide core and resulting in strong fluorescence emission in visible region[55]. Here 4-amino group operates the ICT process to the 1,8-naphthalic anhydride unit and due to ICT effect, 4-aminonaphthalimide unit shows fluorescence emission at 535 nm. Herein, to reduce the ICT effect was reduced because of the presence of electron withdrawing carbamate group with the amino group. But in the presence of tyrosinase, 3-hydroxybenzyl alcohol oxidized into o-dihydroxy derivative which eliminates the free 4-aminonaphthalimidefluorophore which shows emission at 535 nm (Scheme 12) along with gradual quenching of emission at 467 nm. Thus, the synthesized probe P-10serves as a ratiometric fluorescence probe for the quantitative evaluation of tyrosinase activity. The probe, P-10 shows high selectivity and sensitivity to tyrosinase with a detection limit of 0.2 U mL^{-1}.



Scheme 12. Schematic illustration of tyrosinase detection strategy of the probe P-10. Adapted with permission from ref. 33. Copyright © 2018, Royal Society of Chemistry.

For monitoring the endogenous activity of the probe P-10, murine melanoma A375 cells were chosen. Cytotoxicity test of the probe P-10 was done by MTT assay which showed that more than 85% cells retained their viability after interaction with 10μM of P-10. Figure 9A displayed that A375 cells itself have almost zero background fluorescence signal. After incubation for 30 min, cells exhibited the blue emission and no fluorescence from the green channel implies that no tyrosinase activity in cytoplasm of cells took place (Figure 9B). However after 3 h of incubation strong green fluorescence emission observed while blue channel emission significantly reduced, (Figure 9C). Thus, ratiometric responses of P-10 in emission in presence of A375 cells were recorded. Again, the kojic acid treatment experiment confirms that the change in fluorescence emission is coming from probe P-10 after tyrosinase interaction, (Figure 9D).

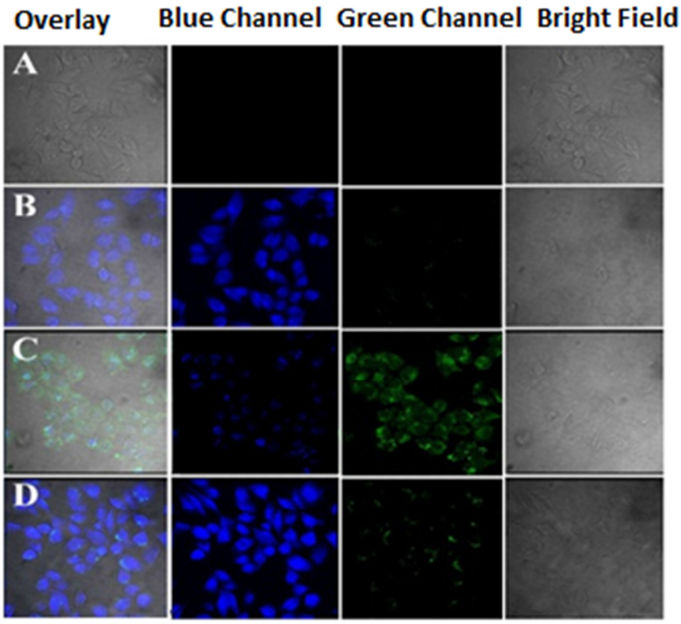
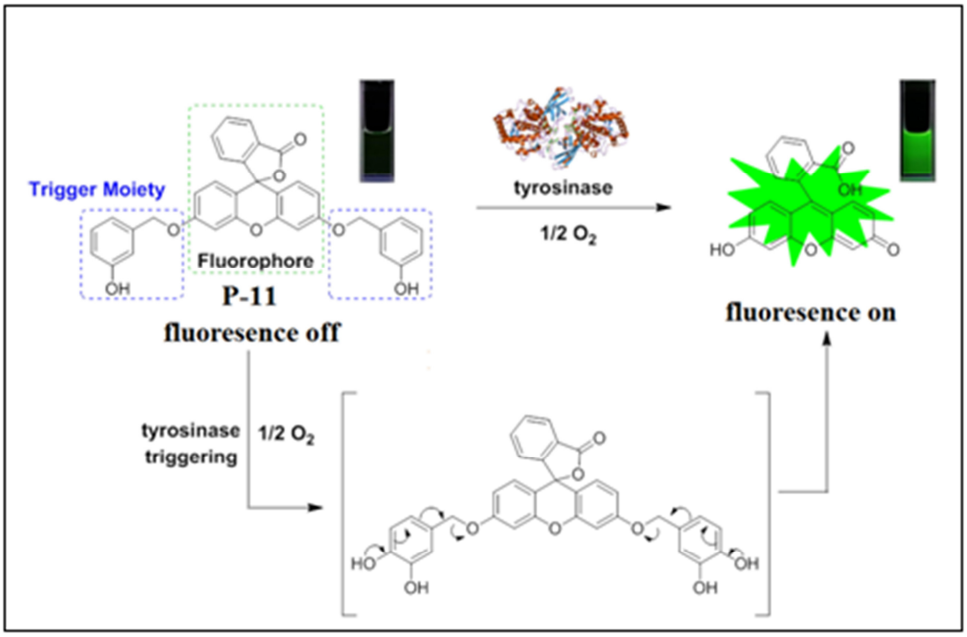


Figure 9. Fluorescence images of A375 cells (A) Control sample (B) Fluorescence images of A375 after 30 min of incubation with probe P-10. Cells show strong blue emission compared to green channel suggested that probe P-10 does not significantly catalyzed by endogenous tyrosinase (C) After 3 h of incubation, green emission of A375 enhanced prominently while blue emission quenched significantly. Green emission indicates the endogenous catalysis of probe P-10. (D) A375 cells were first treated with kojic acid (1mM) for 1 h and then with Probe P-10. No significant change in fluorescence emission from blue to green was observed in the cells. Adapted with permission from ref. 33. Copyright © 2018, Royal Society of Chemistry.

Very recently Hu et al.[56] designed a novel turn-on fluorescent probe, P-11, for highly chemoselective detection of tyrosinase in living cells. In this probe, 3- hydroxyl benzyloxy unit used as the recognition part which influences the hydroxylation reaction at the 4- position by tyrosinase rather than other reactive oxygen species. The fluorescein moiety in the probe P-11 act as fluorophore unit. Quaternary carbon breaks the π-conjugation of the xanthene moiety and makes the probe non-fluorescence. But after incubation with tyrosinase in presence of O_2, hydroxylation occurs at 4-position followed by 1, 6-rearrangement-elimination reaction that causes 24 fold fluorescence enhancement of the fluorophore (Scheme 13). Optical properties of P-11 revealed that the probe shows a broad absorption band centred at 460 nm with a weak emission at 517 nm (Φ = 0.031). But after incubation with tyrosinase, a new absorption occurs at 470 nm associated with a clear color change from colorless to slightly green and emit with a strong fluorescent intensity at 517 nm and in this way a fluorescence off- on the system generated.



Scheme 13. Proposed mechanism of tyrosinase sensing by the probe P-11. Adapted with permission from ref. 56. Copyright © 2019, Elsevier.

Again P-11 shows excellent permeability towards cell-membrane and tissue and this property makes the probe P-11 suitable in tracing endogenous tyrosinase activity in different living cells and zebrafish models. As shown in Figure 10A, zebrafish exhibit almost no background fluorescence in green channel, but after incubating with P-11 (10 μM) for 2 h, a green fluorescence emission distributed throughout the whole body of zebrafish was seen (Figure 10B). After 4 h, the fluorescence signal became stronger in the digestive system that informs higher levels of tyrosinase activity in this part (Figure 10C). Additionally, as shown in Figure 10D, the inhibition experiment with kojic acid proved that the fluorescence comes because of the presence of tyrosinase in zebrafish and thus the probe P-11 can successfully recognize endogenous tyrosinase in living cells.

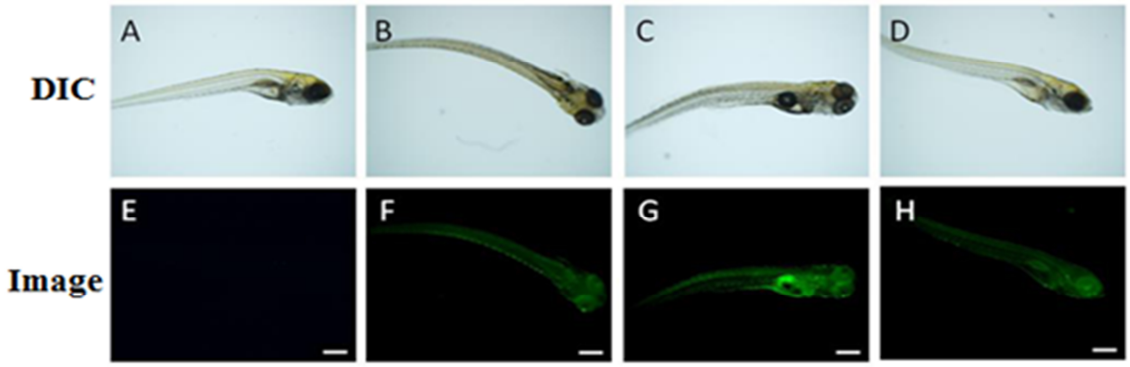


Figure 10. Fluorescence images of zebrafish larvae: (A, E) zebrafish only, (B, F) zebrafish incubated with P-11 (10μM) for 2 h, (C, G) zebrafish incubated with P-11 (10 μM) for 4 h, (D, H) zebrafish pretreated with 500 μM kojic acid for 2 h and then incubated with P-11 (10 μM) for 4 h. Scale bar = 200 μm. Adapted with permission from ref. 56. Copyright © 2019, Elsevier.

4. CONCLUSIONS

In last five years a significant advancement has been achieved in the development of single molecule fluorescent probes for the sensing of tyrosinase activity because of its high sensitivity, easy tunability, finer bioimaging capacity, superior spatiotemporal resolution and noninvasive nature in imaging and detection of biological analytes in the fluorescent analysis. In this article, fluorescent probes based on small organic molecules having a recognition unit for tyrosinase activity and fluorophore units for signaling are thoroughly explained. Again small-molecule fluorescent probes because of their cell permeability can easily penetrate into cells and hence can recognize and image unusual levels of tyrosinase in living cells. Thus a single molecule fluorescent probe could be successfully used as a powerful tool for early detection and diagnosis of unusual levels of tyrosinase activity during cancer growth. In this review, recent advances in this growing field, including synthesis strategies, mechanistic paths and potential applications especially in the biological field are well discussed. In spite of significant progress in the advancement of fluorescent probes for the sensing of tyrosinase activity, some challenges still exist for practical applications. Sensitivity and selectivity aspects are the supreme criteria for accurate detection in early-stage diagnoses which may decrease mortality rate. Efforts should be given to reduce background fluorescence. Again for outstanding biological application specially for endogenous detection and imaging, probes should be NIR or two-photon excitation active because of their low background fluorescence signal and deep penetration strength. Moreover, probes should be soluble in cell medium, have sufficient stability, high cell permeability and low cytotoxicity for in vivo sensing and imaging of tyrosinase activity. Thus, this area still needs further studies for proper application in clinics and healthcare systems.

5. CONFLICT OF INTEREST

Conflict of interest declared none

6. REFERENCES

1. Rolff MA, Schottenheim JU, Decker HE, Tuczek FE. Copper–O_2 reactivity of tyrosinase models towards external monophenolic substrates: molecular mechanism and comparison with the enzyme. Chem. Soc. Rev. 2011, 40 (7): 4077-4098. DOI: 10.1039/C0CS00202J
2. Briganti ST, Camera EM, Picardo MA. Chemical and instrumental approaches to treat hyperpigmentation. Pigm. Cell Res. 2003; 16 (2): 101-110. DOI: 10.1034/j.1600-0749.2003.00029.x
3. Solem EV,Tuczek FE, Decker HE. Tyrosinase versus catechol oxidase: One asparagine makes the difference. Angew. Chem. Int. Ed. 2016; 55 (8): 2884-2888. DOI: 10.1002/anie.201508534
4. Tessari IS, Bisaglia MA, Valle FR, Samori BR, Bergantino EL, Mammi ST, et al. The reaction of α-synuclein with tyrosinasehas possible implications for parkinson disease. J. Biol. Chem. 2008; 283: 16808-16817. DOI: 10.1074/jbc.M709014200
5. Kim TA, Park JI, Park SE, Choi YO, Kim YO. Visualization of tyrosinase activity in melanoma cells by a BODIPY-based fluorescent probe. Chem. Commun. 2011; 47 (47): 12640-12642. DOI: 10.1039/C1CC15061H
6. Kim JI, Kim YO. A water-soluble sulfonate-BODIPY based fluorescent probe for selective detection of HOCl/OCl− in aqueous media. Analyst. 2014; 139 (12): 2986-2989. DOI: 10.1039/C4AN00466C

7. Battaini GI, Monzani EN, Casella LU, Lonardi EM, Tepper AR, Canters GE, et al. Tyrosinase-catalyzed oxidation of chlorophenols. J. Biol. Chem. 2002; 277: 44606-44612.DOI: 10.1074/jbc.M207829200
8. Fiddler IS, Melanoma Metastasis. Cancer Control. 1995; 2 (5): 398-404. DOI: 10.1177/107327489500200503
9. Shah S, Raheem N, Daud S, Mubeen J, Shaikh A, Baloch A, et al. Mutational spectrum of the TYR and SLC45A2 genes in Pakistani families with oculocutaneous albinism, and potential founder effect of missense substitution of tyrosinase. J. Clin. Exp. Dermatol. 2015; 40: 774-780. DOI: 10.1111/ced.12612
10. Gray-Schopfer VA, Wellbrock CL, Marais RI. Melanoma biology and new targeted therapy.Nature.2007; 445: 851-857. DOI: 10.1038/nature05661
11. Qin HU, Shang ZH, Jantan IB, Tan OY, Hussain MU, Sher MU, et al. Molecular docking studies and biological evaluation of chalcone based pyrazolines as tyrosinase inhibitors and potential anticancer agents. RSC Adv. 2015; 5 (57): 46330-46338. DOI: 10.1039/C5RA02995C
12. Ando HI, Kondoh HI, Ichihashi MA, Hearing VI. Approaches to Identify Inhibitors of Melanin Biosynthesis via the Quality Control of Tyrosinase. J. Invest. Dermatol. 2007; 127 (4): 751-761. DOI: 10.1038/sj.jid.5700683
13. Liu XI, Wang FU, Niazov-Elkan AN, Guo WE, Willner IT. Probing biocatalytic transformations with luminescent dna/silver nanoclusters. Nano Lett. 2013; 13 (1): 309-314.DOI: 10.1021/nl304283c
14. Lei CU, Zhao XI, Sun JI, Yan XI, Gao YU, Gao HA, et al. A simple and novel colorimetric assay for tyrosinase and inhibitor screening using 3,3′,5,5′-tetramethylbenzidine as a chromogenic probe. Talanta.2017; 175: 457-462. DOI: 10.1016/j.talanta.2017.07.070
15. Jiang CH, Li YA, Liu CH, Qiu LI, Li ZH. A general and versatile fluorescence turn-on assay for detecting the activity of protein tyrosine kinases based on phosphorylation-inhibited tyrosyl oxidation. Chem. Comm. 2016; 52 (85): 12570-12573. DOI: 10.1039/C6CC07035C
16. Li DI, Gill RO, Freeman RO, Willner IT. Probing of enzyme reactions by the biocatalyst-induced association or dissociation of redox labels linked to monolayer-functionalized electrodes. Chem. Comm. 2006; 48: 5027-5029. DOI: 10.1039/B614141B
17. 17. Gill RO, Freeman RO, J-P Xu, Willner IT, Winograd SH, Shweky IT, et al. Probing biocatalytic transformations with CdSe-ZnS QDs. J. Am. Chem. Soc. 2006; 128 (48): 15376-15377. DOI: 10.1021/ja066636t
18. Li HU, Cao ZH, Zhang YU, Lau CH, Lu JI. Simultaneous detection of two lung cancer biomarkers using dual-color fluorescence quantum dots. Analyst. 2011; 136 (7): 1399-1405. DOI: 10.1039/C0AN00704H
19. He HU, Xie CH, Ren JI. Non bleaching fluorescence of gold nano particles and its applications in cancer cell imaging. J. Anal. Chem. 2008; 80 (15): 5951-5957. DOI: 10.1021/ac8005796
20. Hettiarachchi SU, Prasai BI, McCarley RO. Detection and cellular imaging of human cancer enzymes using a turn-on, wavelength-shiftable, self-immolativeprofluorophore. J. Am. Chem. Soc. 2014; 136 (21): 7575-7578. DOI: 10.1021/ja5030707
21. Wu DI, Ryu JA, Chung YO, Lee DA, Ryu JI, Yoon JO, et al. A far-red-emitting fluorescence probe for sensitive and selective detection of peroxynitrite in live cells and tissues. J. Anal. Chem. 2017; 89 (20): 10924-10931. DOI: 10.1021/acs.analchem.7b02707
22. Chen HA, He XI, Su ME, Zhai WE, Zhang HA, Li CH. A general strategy toward highly fluorogenic bioprobes emitting across the visible spectrum. J. Am. Chem. Soc. 2017; 139 (29): 10157-10163. DOI: 10.1021/jacs.7b05920
23. Beck MI, Kathayat RA, Cham CA, Chang EU, Dickinson BR. Michael addition-based probes for ratiometric fluorescence imaging of protein S-depalmitoylases in live cells and tissues. Chem. Sci. 2017; 8 (11): 7588-7592. DOI: 10.1039/C7SC02805A
24. Zhou JI, Shi WE, Li LI, Gong QI, Wu XI, Li XI, Ma HU. Detection of maldistribution of tyrosinase from melanosomes to lysosomes and its upregulation under Psoralen/Ultraviolet A with a melanosome-targeting tyrosinase fluorescent probe.Anal.Chem. 2016; 88 (8): 4557-4564.DOI: 10.1021/acs.analchem.6b00742
25. Wu XI, Li XI, Li HO, Shi WE, Ma HU. A highly sensitive and selective fluorescence off-on probe for the detection of intracellular endogenous tyrosinase activity. Chem. Commun. 2017; 53 (16): 2443-2446. DOI: 10.1039/C6CC09679D
26. Li HU, Liu WEN, Zhang FE, Zhu XI, Huang LI, Zhang HA. Highly selective fluorescent probe based on hydroxylation of phenylboronic acid pinacol ester for detection of tyrosinase in cells. Anal. Chem. 2018; 90 (1): 855-858. DOI: 10.1021/acs.analchem.7b03681
27. Kim TA, Park JI, Park SE, Choi YO, Kim YO. Visualization of tyrosinase activity in melanoma cells by a BODIPY-based fluorescent probe. Chem. Commun. 2011; 47: 12640-12642. DOI: 10.1039/C1CC15061H
28. Bobba KO, Won MI, Shim IN, Velusamy NI, Yang ZH, Qu JU. et al. A BODIPY-based two-photon fluorescent probe validates tyrosinase activity in live cells. Chem. Commun. 2017; 53 (81): 11213-11216. DOI: 10.1039/C7CC05043G
29. Yan SH, Huang RO, Wang CH, Zhou YI, Wang JI, Fu BO, et al. A two-photon fluorescent probe for intracellular detection of tyrosinase activity. Chem. Asian J. 2012; 7: 2782-2785. DOI: 10.1002/asia.201200762
30. Li ZH, Wang YI, Zhang XU, Zeng CH, Hu LI, Liang XI. A tyrosinase-triggered oxidative reaction-based "Turn-on" fluorescent probe for imaging in living melanoma cells. Sens. Actuators, B. 2017; 242: 189-194. DOI: 10.1016/j.snb.2016.11.011
31. Qiu BO, Zeng YI, Cao LI, Hu RU, Zhang XI, Yu TI, et al. A colorimetric and ratiometric fluorescence sensor for sensitive detection of fluoride ions in water and toothpaste. RSC Adv. 2016; 6 (54): 49158-49163. DOI: 10.1039/C6RA04747E
32. Han XI, Song XI, Yu FA, Chen LI. A ratiometric near-infrared fluorescent probe for quantification and evaluation of selenocysteine-protective effects in acute inflammation. Adv. Funct. Mater. 2017; 27 (28): 1700769. DOI: 10.1002/adfm.201700769
33. Sidhu JA, Singh AS, Garg NE, Kaur NA, Singh NA. A highly selective naphthalimide-based ratiometric

fluorescent probe for the recognition of tyrosinase and cellular imaging. Analyst. 2018; 143 (18): 1853-1861. DOI: 10.1039/C8AN01136B

34. Mondal SA, Rakshit AN, Pal SU, Datta AN. Cell permeable ratiometric fluorescent sensors for imaging phosphoinositides. ACS Chem. Biol. 2016; 11 (7): 1834-1843. DOI: 10.1021/acschembio.6b00067
35. Wu W, Wang Z, Dai X, Miao J, Zhao B. An effective colorimetric and ratiometric fluorescent probe based FRET with a large Stokes shift for bisulfate. Sci. Rep. 2016; 6: 25315. DOI: 10.1038/srep25315
36. Zhang HO, Liu JI, Sun YU, Huo YI, Li YA, Liu WEN, et al. A mitochondria-targetable fluorescent probe for peroxynitrite: fast response and high selectivity. Chem. Comm. 2015; 51 (13): 2721-2724. DOI: 10.1039/C4CC09122A
37. Zhao CH, An JI, Zhou LI, Fei QI, Wang FE, Tan JI, et al. Transforming the recognition site of 4-hydroxyaniline into 4-methoxyaniline grafted onto a BODIPY core switches the selective detection of peroxynitrite to hypochlorous acid. Chem. Comm. 2016; 52 (10): 2075-2078. DOI: 10.1039/C5CC08936K
38. Wu XI, Li XI, Li HO, Shi WE, Ma HU. A highly sensitive and selective fluorescence off-on probe for the detection of intracellular endogenous tyrosinase activity. Chem. Commun. 2017; 53 (16), 2443-2446. DOI: 10.1039/C6CC09679D
39. Peng MA, Wang YA, Fu QI, Sun FE, Na NA, Ouyang JI. Melanosome-targeting near-infrared fluorescent probes with large stokes shift for in situ quantification of tyrosinase activity and assessing drug effects on differently invasive melanoma cells. Anal. Chem. 2018; 90 (10): 6206-6213. DOI: 10.1021/acs.analchem.8b00734
40. Yu CH, Li XI, Zeng FA, Zheng FA, Wu SH. Carbon-dot-based ratiometric fluorescent sensor for detecting hydrogen sulfide in aqueous media and inside live cells, Chem. Commun. 2013; 49 (4): 403-405. DOI: 10.1039/C2CC37329G
41. Yang XI, Luo YA, Zhuo YA, Feng YU, Zhu SH. Novel synthesis of gold nano clusters templated with l-tyrosine for selective analyzing tyrosinase. Anal. Chim. Acta. 2014; 840: 87-92. DOI:10.1016/j.aca.2014.05.050
42. Sun JU, Mei HA, Wang SU, Gao FE. Two-photon semiconducting polymer dots with dual-emission for ratiometric fluorescent sensing and bio-imaging of tyrosinase activity. Anal. Chem. 2016; 88 (14): 7372-7377. DOI: 10.1021/acs.analchem.6b01929
43. Chen Y. Advances in fluorescent probes for detection and imaging of endogenous tyrosinase activity. Anal Biochem. 2020; 594(1): 113614

43a. Nawaz AL, Shafi TA, Khaliq AB, Mukhtar HA, Haq IK. Tyrosinase: Sources, Structure and Applications. Int J Biotech & Bioeng. 2017; 3 (5): 142-148. DOI: 10.25141/2475-3432-2017-5.0135

44. Zhipeng LI, Wang YI, Zeng CH, Hu LI, Liang XI. Ultra-sensitive tyrosinase-activated turn-on near-infrared fluorescent probe with a rationally designed urea bond for selective imaging and photodamage to melanoma cells. Anal. Chem. 2018; 90 (6): 3666-3669. DOI:10.1021/acs.analchem.7b05369
45. Lo JC, Darracq MA, Clark RI. A review of methylene blue treatment for cardiovascular collapse. J. Emerg. Med. 2014; 46 (5): 670-679. DOI:10.1016/j.jemermed.2013.08.102
46. Jordan AM, Khan TH, Malkin H, Osborn HM, Photiou A, Riley PA. Melanocyte-directed enzyme prodrug therapy (MDEPT): development of second generation prodrugs for targeted treatment of malignant melanoma. Bioorg. Med Chem. 2001; 9 (6): 1549-1558.DOI: 10.1016/s0968-0896(01)00039-6
47. Zhan CH, Cheng JI, Li BO, Huang SH, Zeng FA, Wu SH. A fluorescent probe for early detection of melanoma and its metastasis by specifically imaging tyrosinase activity in a mouse model. Anal. Chem. 2018; 90 (15): 8807-8815. DOI: 10.1021/acs.analchem.8b00594
48. Zhang PE, Li SH, Zhang QI, Xiao YU, Ding CA. A colorimetric and near-infrared ratiometric fluorescent probe for the determination of endogenous tyrosinase activity based on cyanine aggregation. Analyst. 2019; 144 (18): 5472-5478. DOI: 10.1039/C9AN01045A
49. Li QI, Yan CH, Guo ZH, Zhu WE. Ratiometric and light-up near-infrared fluorescent DCM-based probe for real-time monitoring endogenous tyrosinase activity. Dyes and Pigments.2019; 162: 802-807. DOI: 10.1016/j.dyepig.2018.11.019
50. Wang JI, Qian JI, Teng ZH, Cao TI, Gong DE, Liu WEN, Yuping Cao, et al. Self-assembling ratiometric fluorescent micelle nanoprobe for tyrosinase detection in living cells. ACS Appl. Nano Mater. 2019; 2(6): 3819-3827. DOI: 10.1021/acsanm.9b00689
51. Wang CH, Yan SH, Huang RO, Feng SH, Fu BO, Weng XI, Zhou XI. A turn-on fluorescent probe for detection of tyrosinase activity. Analyst. 2013; 138 (10): 2825-2828.DOI: 10.1039/C3AN00272A
52. Xiaofeng WU, Lihong LI, Wen SH, Qiuyu GO, Huimin MA. Near-infrared fluorescent probe with new recognition moiety for specific detection of tyrosinase activity: Design, synthesis, and application in living cells and zebrafish. AngewandteChemie. 2016; 55(47): 14728-14732. DOI: 10.1002/anie.201609895
53. Zhou JI, Ma HU. Design principles of spectroscopic probes for biological applications. Chem. Sci. 2016; 7 (10): 6309-6315. DOI: 10.1039/C6SC02500E
54. Li HU, Liu WEN, Zhang FE, Zhu XI, Huang LI, Zhang HA. Highly selective fluorescent probe based on hydroxylation of phenylboronic acid pinacol ester for detection of tyrosinase in cells. Anal. Chem. 2018; 90 (1): 855-858. DOI: 10.1021/acs.analchem.7b03681
55. Thavornpradit SO, Sririrak JI, Wanichacheva NA, Turn-on naphthalimide fluorescent sensor with high quantum yield and large Stokes shift for the determination of Cu(II). J. Photochem. Photobiol.A : Chemistry. 2016; 330: 55-63. DOI: 10.1016/j.jphotochem.2016.07.010
56. Hu SI, Wanga TI, Zoua JI, Zhou ZH, Lu CU, Nie JU, et al. Highly chemoselective fluorescent probe for the detection of tyrosinase in living cells and zebrafish model. Sensors and Actuators B: Chemical. 2019; 283(15): 873-880. DOI:10.1016/j.snb.2018.12.105