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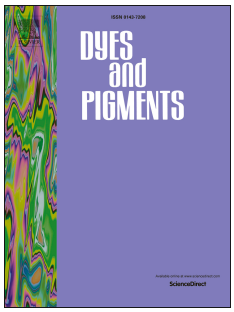

BODIPY-based fluorescent probes for mitochondria-targeted cell imaging with superior brightness, low cytotoxicity and high photostability

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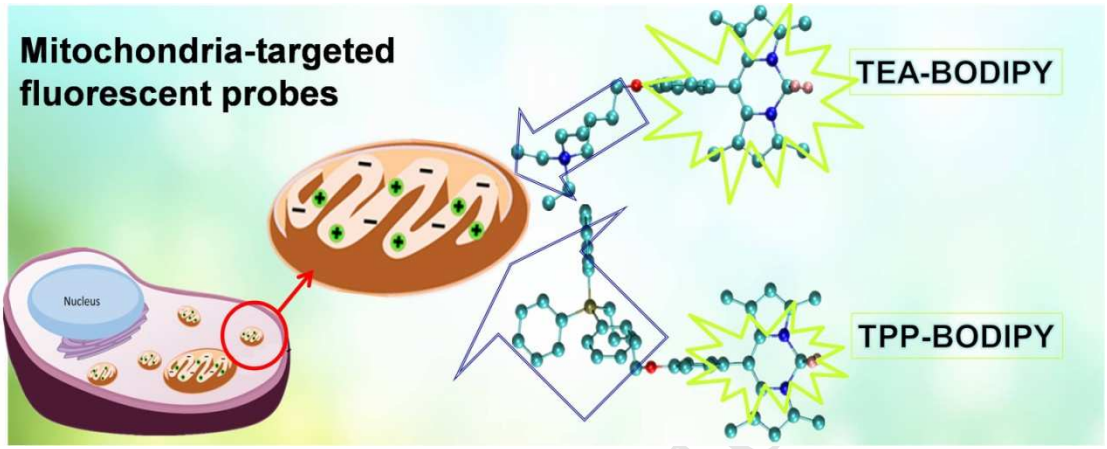
Graphical abstract

Two BODIPY-based fluorescent probes can specifically stain mitochondria with superior brightness, low cytotoxicity and high photostability.



Short communication

BODIPY-based fluorescent probes for mitochondria-targeted cell imaging with superior brightness, low cytotoxicity and high photostability

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Abstract:

Two mitochondria-targeted fluorescent probes (TPP- and TEA-BODIPY) were rationally designed and easily synthesized. These probes can specifically stain mitochondria in living cells with superior brightness, low cytotoxicity and high photostability, thus are quite promising as mitochondrial tracking probes.

Keywords: Fluorescent probe；mitochondria；imaging；photostability

1. Introduction

Mitochondria, the important organelles in almost all eukaryotic cells, play a crucial role in a number of vital cellular processes, including energy generation, central metabolism, redox signalling, calcium modulation and apoptotic pathways [1-3]. Recent discoveries showed that mitochondrial morphology was closely associated with cell functions [4-7]. When cells are impaired, the size, number and structure of mitochondria often change. For instance, mitochondrial swelling is often observed in damaged cells as a common phenomenon [8,9]. It was also pointed out that mitochondrial dysfunction could induce mitophagy, which led to the change of cell function and several neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease [10,11].

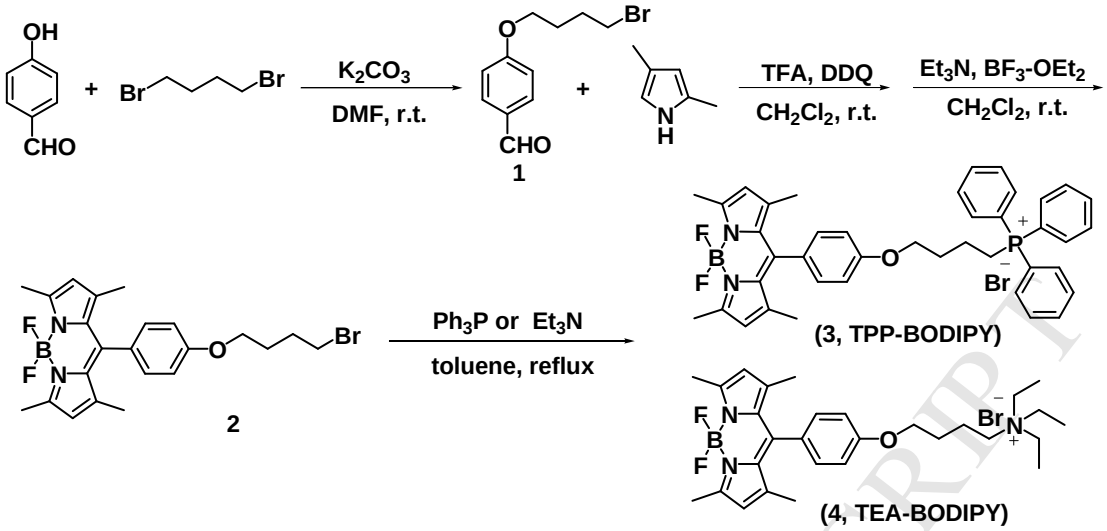
On account of the importance of mitochondria, fluorescent dyes targeting mitochondria have attracted broad interests because of their capability of observing mitochondrial morphology so as to provide useful information for biochemical cellular processes and even early diagnosis of diseases [12,13]. As is well known, a lot of organic dyes have been used in the imaging of mitochondria, such as Rhodamine [14-16], F16 [17,18], MitoTracker@RedCMXRos (**MTR**) and MitoTracker@Green FM (**MTG**). However, most of these fluorescent dyes suffer from severe drawbacks including difficult modification of molecular structure, poor photostability and weak biocompatibility which limit their applications in living cell studies. So, it is still highly necessary and urgent to design mitochondria-targeted fluorescent probes with high affinity and selectivity. Recently, boron-dipyrromethene (BODIPY) derivatives are regarded as one of the most popular family of fluorescent dyes in the field of bioimaging owing to their advantages, *e. g.* large fluorescence quantum yield (QY), easy modification, sharp fluorescence emission, high extinction coefficient and excellent photostability [19-21]. Previous mitochondria-targeted fluorescent dyes mainly utilized delocalized lipophilic cations (DLC) as cargo groups in consideration of the negative mitochondrial membrane potential [22,23]. The most widely used mitochondria-targeted delivery moiety nowadays is cationic triphenylphosphine (TPP^+). However, reports about mitochondria-targeted capability

of a simpler cationic triethylamine (TEA^+) are sparse. In this paper, we report two novel mitochondria-targeted fluorescent probes, namely **TPP-BODIPY** and **TEA-BODIPY**, by combining the cargo TPP^+ and TEA^+ with the fluorophore BODIPY by a flexible carbon chain, respectively. By this way, the cationic groups can guide BODIPY into mitochondria, while BODIPY still maintains its attractive properties.

2. Results and discussion

2.1. *Synthesis*

The target products were synthesized in three steps (Scheme 1) [24]. First, the reaction of 4-hydroxybenzaldehyde with 1,4-dibromobutane in the presence of K_2CO_3 as a base gave **1** in 86% yield. Second, the aldehyde **1** was reacted with 2, 4-dimethylpyrrole using trifluoroacetic acid (TFA) as a catalyst, followed by the oxidation with 2, 3-dichloro-5, 6-dicyano-1,4-benzoquinone (DDQ) and subsequent complexation with $BF_3.OEt_2$ in the presence of triethylamine (Et_3N), which afforded a highly emissive fluorophore (**2**) in 22% yield. Finally, reflux of **2** with triphenylphosphine (TPP) or triethylamine (TEA) in toluene gave the title product **3** (**TPP-BODIPY**) and **4** (**TEA-BODIPY**) in 46% and 37% yield, respectively. All the products were well characterized by NMR (Fig.S1) and mass spectrometry (Fig.S2). The mass spectroscopic data showed main peaks at *m/z* 496.35 and 657.40, which were attributed to M^+ for **TPP-BODIPY** and **TEA-BODIPY**, respectively, with deviation less than 1% from the theoretical values. **TEA-BODIPY** could be absolutely dissolved in water, while **TPP-BODIPY** could be readily dissolved in water by dilution from a stock solution in dimethyl sulphoxide (DMSO). In all experiments, the solutions contained less than 1% (v/v) DMSO, which was usually allowed in biology-related experiments.



Scheme 1. Synthetic route of **TPP-BODIPY** and **TEA-BODIPY**.

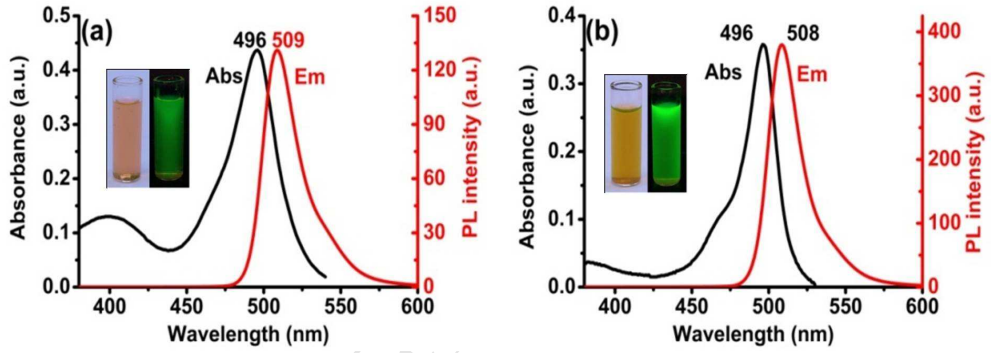


Fig. 1 Absorption and emission spectra of **TPP-BOBIPY** (a) and **TEA-BOBIPY** (b) with a concentration of 5 μM in water. The insets are the photographs of **TPP-BOBIPY** (a) and **TEA-BOBIPY** (b) under ambient (left) and UV light (365 nm, right), respectively.

Table 1 Photophysical properties of **TPP-BODIPY** and **TEA-BODIPY** ([a] Absorption maxima. [b] Emission maxima. [c] Molar extinction coefficient).

Compound (solvent)	λ_{abs} [a] (nm)	λ_{em} [b] (nm)	QY	MEC [c] ($M^{-1}cm^{-1}$)
TPP-BODIPY (H_2O)	496	509	0.49	6.95×10^4
TPP-BODIPY (CH_3CN)	496	509	0.75	7.88×10^4
TEA-BODIPY(H_2O)	496	508	0.69	6.22×10^4
TEA-BODIPY(CH_3CN)	497	510	0.75	7.41×10^4

2.2. *Photophysical properties*

Recent literatures indicated that the microenvironment of mitochondria changes during many cellular processes involved in spatial arrangement and protein composition [25,26]. Thus the *in vivo* imaging capacity of mitochondria-targeted fluorescent dyes is closely related to their stability in surrounding environments. Here, the optical properties of **TPP-BODIPY** and **TEA-BODIPY** in commonly used polar solvents (water and acetonitrile) were studied (Fig. 1 and Table 1). The absorption and emission maxima of **TPP-BODIPY** are centred at 496 nm and 509 nm respectively in water (Fig. 1a). And we can find that the absorption and emission maxima of **TPP-BODIPY** in acetonitrile are consistent with those in water (Fig. S3a in ESI). Moreover, there is a smaller peak at ~ 400 nm which is contributed by triphenylphosphine (Fig. 1a). The results also show that the polarity of different solvents has almost no impact on the optical properties of **TEA-BODIPY** (Fig. 1b and Fig. S3b). These results indicate the probes can be applied in wide conditions. Owing to the non-conjugation between the fluorescent BODIPY core and the cargo group (TPP^+ or TEA^+) *via* a flexible carbon chain, there is no variation in the absorption and emission maxima upon replacement of cargo group TPP^+ by TEA^+, *i.e.* absorption maximum ~ 496 nm and emission maximum ~509 nm. In addition, the fluorescence intensity of **TEA-BODIPY** is significantly stronger than that of **TPP-BODIPY** both in water and acetonitrile (Fig. 1 and Fig. S3). Besides, the molar extinction coefficients for both probes in water and acetonitrile are in the range of 6 – 8 $\times 10^4$ M^{-1} cm^{-1}, demonstrating BODIPY is an ideal candidate as a light absorbing material in broad application fields.

BODIPY is a commonly used fluorophore, but it encounters some troubles when applied in biological system due to its poor water solubility [27,28]. To solve this problem, many researchers modified BODIPY with some water-soluble groups [29-31]. However, the QY of BODIPY would inevitably decrease during the modification [32-35]. In this work, the two probes have very good water solubility. More importantly, the QY of the two probes in water were 0.49 and 0.69 (Fig. S4, using Rhodamine123 as reference [36]), respectively, which are apparently larger than

those of the BODIPY-based water-soluble probes reported previously [27-35]. Additionally, the larger QY of **TEA-BODIPY** than that of **TPP-BODIPY** indicated the former might be more excellent than the latter for living cell imaging. Overall, the excellent water solubility and fluorescence properties demonstrated the tremendous application prospect of these two probes.

2.3. *Stability of the probes under different pH*

To our knowledge, mitochondria present weak alkaline environment in living cells [37-39]. Eligible mitochondria-targeted fluorescent probes must be able to work in a mitochondrial physiology environment. Experimental results show that the two probes exhibit high fluorescence intensity in the pH range from 2 – 10 (Fig. 2), so they are suitable for both acid and basic conditions.

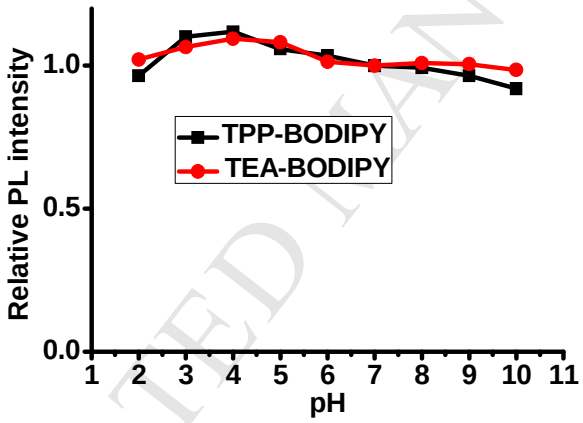


Fig. 2 pH dependent fluorescence intensity of **TPP-BOBIPY** (5 μM) and **TEA-BOBIPY** (5 μM).

2.4. *Cytotoxicity*

Low cytotoxicity is one of the most critical requirements for fluorescent probes. The cytotoxicity of **TPP-BODIPY** and **TEA-BODIPY** were evaluated using a standard MTT [3-(4, 5-dimethylthiazol)-2, 5-diphenyltetrazolium bromide] method. Two cell lines, cervical carcinoma (HeLa) and gastric cancer (SGC-7901) cell lines, were incubated with **TPP-BODIPY** and **TEA-BODIPY** at a concentration range from 0 to 200 μM for 48 h, and the cytotoxic effects of the two probes are shown in Fig. 3 and Fig. S5.

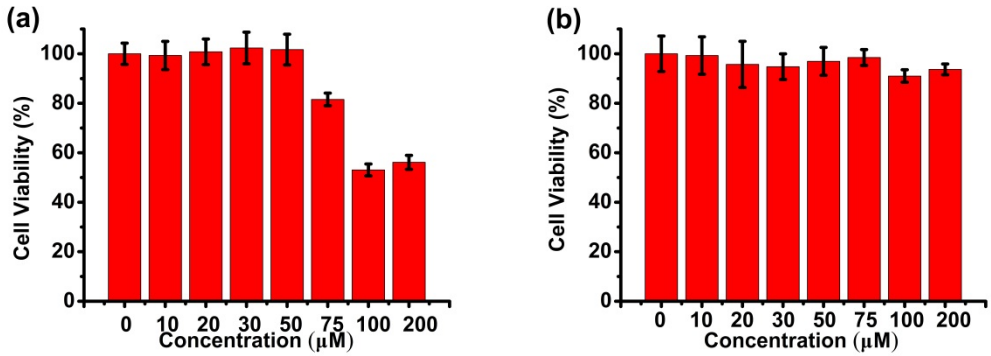


Fig. 3 Cell viability of Hela cells treated with different concentrations of **TPP-BODIPY** (a) and **TEA-BODIPY** (b) for 48 h.

It clearly shows that those two fluorescent probes exhibit no obvious toxicity towards carcinoma and normal cell lines at low concentrations. For instance, over half of the Hela cells are still alive after treatment with 100 μM of **TPP-BODIPY** for 48 h (Fig. 3a). In fact, cells for bioimaging were exposed to a quite low concentration (0.5 μM) of the probes. Notably, **TEA-BODIPY** shows much less cytotoxicity than **TPP-BODIPY** even at relatively high concentration (200 μM, Fig. 3b). The results suggest that **TEA-BODIPY** may be more suitable for imaging of living cells than **TPP-BODIPY** in consideration of the quite low cytotoxicity and high QY (0.69).

2.5. *Confocal microscopic imaging*

To investigate the imaging performance of **TPP-BODIPY** and **TEA-BODIPY** in HeLa cells, the confocal microscopic imaging experiment were conducted by using a Nikon A1 Confocal laser scanning microscope. As shown in Fig. 4, **TPP-BODIPY** and **TEA-BODIPY** display bright green fluorescence in HeLa cells, owing to their really high quantum yields，and give no fluorescence in the red channel (Fig. S6). From co-localization images of the fluorescent probes with Mito Tracker Red (**MTR**), it can be observed that the green fluorescence of **TPP-BODIPY** and **TEA-BODIPY** is perfectly overlapped with the red fluorescence of **MTR** (Figs. 4c and 4g). The Pearson correlation coefficients, which are used to quantify the overlap between the fluorescence of **MTR** and that of **TPP-BODIPY** or **TEA-BODIPY**, are calculated as 0.87 and 0.90. As depicted in Figs. 4d and 4h**,** the changes in the fluorescence intensity profiles of linear regions of interest (ROIs) are synchronous for

TPP-BODIPY (or **TEA-BODIPY**) and **MTR**, indicating that **TPP-BODIPY** and **TEA-BODIPY** can selectively accumulate in mitochondria of living cells. Moreover, the fluorescent images of cells incubated with **TEA-BODIPY** are brighter than those of **TPP-BODIPY**, which could be ascribed to the higher QY of **TEA-BODIPY** (0.69) than **TPP-BODIPY** (0.49).

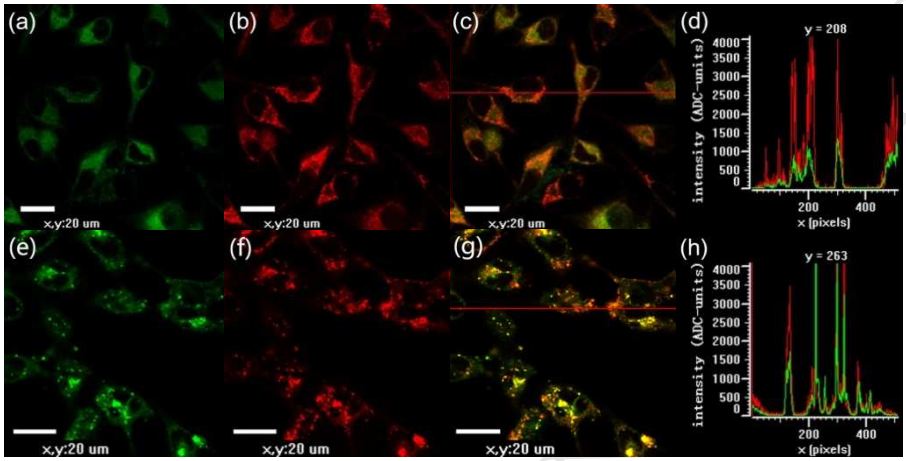


Fig. 4 Fluorescent images of HeLa cells stained with 0.5 μM **TPP-BODIPY** (a) or 0.5 μM **TEA-BODIPY** (e) for 4 h, 0.5 μM **MTR** for 15 min (b, f). Panel c and g are the overlap of (a) and (b), (e) and (f), respectively. Panel d and h are the fluorescence intensity profiles of ROIs across HeLa cells. Green lines represent the intensity of the probe and red lines represent the intensity of **MTR**. Excitation wavelength: 488 nm for **TPP-BODIPY** and **TEA-BODIPY**, and 543 nm for **MTR**.

2.6. *Cellular uptake*

The cellular uptake of the mitochondria-targeted fluorescent probes by HeLa cells was examined by flow cytometry. HeLa cells were incubated with 0.5 μM **TPP-BODIPY** or 0.5 μM **TEA-BODIPY** for different times, and then the uptake content was surveyed with the intrinsic fluorescence of probes. The uptake rates for both probes are quite fast, and over 80% probes are internalized within 1 h (Fig. 5). Additionally, the fluorescence intensity for **TEA-BODIPY** is higher than that of **TPP-BODIPY** as observed by flow cytometry, which is consistent with their quantum yields. With this merit, the staining with **TEA-BODIPY** will necessitate a shorter incubation time and improved efficiency of the mitochondrial imaging.

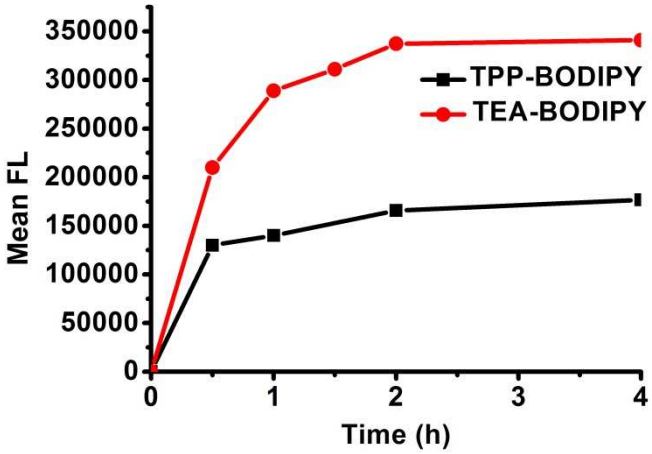


Fig. 5 Flow cytometric analysis of the cellular uptake of **TPP-BODIPY** (0.5 μM) and **TEA-BODIPY** (0.5 μM) by HeLa cells.

2.7. *Photostability*

Photostability of fluorescent probe is one of the major concerns when applied for real-time imaging of living cells [40,41]. The photostability of **TPP-BODIPY** and **TEA-BODIPY** were measured by a Carl Zeiss LSM 710 laser confocal microscope with an excitation energy of 65 μW. There is almost no loss of fluorescence signal of **TPP-BODIPY** after irradiation for 218 s (Figs. 6a and S7). **TEA-BODIPY** shows good photostability (Figs. 6b and S8) with small loss of signal due to the minor shrinkage of the target cell (Fig. 6b), which results in less collection of fluorescence signal, rather than the photobleaching of **TEA-BODIPY**. In contrast, the fluorescence image of commercial **MTR** cannot be observed after irradiation for only 136 s (Figs. 6c and S9), and the fluorescence signal decreases by more than 80% after irradiation (Fig. 6d). It demonstrates that **MTR** possesses poor stability under the irradiation, as reported by previous study [42,43]. So, **TPP-BODIPY** and **TEA-BODIPY** are expected to have better performances than **MTR** for long-time tracking in living cells because of their excellent photostability.

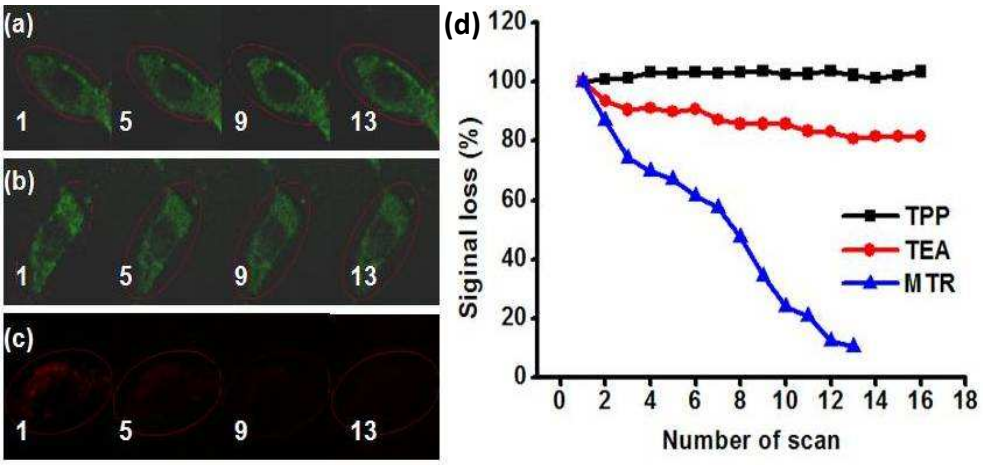


Fig. 6 Photobleaching experiments of 0.5 μM **TPP-BODIPY** (a), 0.5 μM **TEA-BODIPY** (b) and 0.5 μM **MTR** (c) in HeLa cells with irradiation for 1, 5, 9 and 13 times. The right column (d) shows the loss of fluorescence signal (%) with increasing irradiation times.

3. Conclusion

In conclusion, two fluorescent probes, namely **TPP-BODIPY** and **TEA-BODIPY**, were successfully synthesized and applied to mitochondria-targeted imaging in living cells. They possessed many advantages, including superior brightness, high photostability, low cytotoxicity and fast internalization, thus they are quite promising as candidates for mitochondria-specific imaging. In addition, compared with the commonly used mitochondria-targeted functional group (*e.g.* TPP^+), TEA^+ has smaller chemical structure, better water solubility and biocompatibility, it may provide new strategies for the future design and fabrication of highly efficient mitochondria-targeted fluorescent probes.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

References

[1] S. J. Chen, Y. N. Hong, Y. Liu, J. Z. Liu, C. W. T. Leung, M. Li, R. T. K. Kwok, E. G. Zhao, J. W. Y. Lam, Y. Yu, B. Z. Tang, Full-range intracellular pH sensing by an aggregation-induced emission-active two-channel ratiometric fluorogen, J. Am. Chem. Soc., 135 (2013), pp. 4926-4929.

[2] J. Estaquier, D. Arnoult, Inhibiting Drp1-mediated mitochondrial fission selectively prevents the release of cytochrome c during apoptosis, Cell Death Differ., 14 (2007), pp. 1086-1094.

[3] H. M. McBride, M. Neuspiel, S. Wasiak, Mitochondria: more than just a powerhouse, Current Biology, 16 (2006), pp. R551-R560.

[4] M. Karbowski, R. J. Youle, Dynamics of mitochondrial morphology in healthy cells and during apoptosis, Cell Death Differ., 10 (2003), pp. 870-880.

[5] S. C. Dodani, S. C. Leary, P. A. Cobine, D. R. Winge, C. J. Chang, A targetable fluorescent sensor reveals that copper-deficient SCO1 and SCO2 patient cells prioritize mitochondrial copper homeostasis, J. Am. Chem. Soc., 133 (2011), pp. 8606-8616.

[6] P. Dong, J. H. Li, S. P. Xu, X. J. Wu, X. Xiang, Q. Q. Yang, J. C. Jin, Y. Liu, F. L. Jiang, Mitochondrial dysfunction induced by ultra-small silver nanoclusters with a distinct toxic mechanism, J. Hazard. Mater., 308 (2016), pp. 139-148.

[7] L. Y. Yang, J. L. Gao, T. Gao, P. Dong, L. Ma, F. L. Jiang, Y. Liu, Toxicity of polyhydroxylated fullerene to mitochondria, J. Hazard. Mater., 301 (2016), pp. 119-126.

[8] J. S. Lee, Y. K. Kim, M. Vendrell, Y. T. Chang, Diversity-oriented fluorescence library approach for the discovery of sensors and probes, Mol. Biosyst., 5 (2009), pp. 411-421.

[9] J. Hayashi, M. Takemitsu, Y. Goto, I. Nonaka, Human mitochondria and mitochondrial genome function as a single dynamic cellular unit, J. Cell Biol., 125 (1994), pp. 43-50.

[10] G. Masanta, C. H. Heo, C. S. Lim, S. K. Bae, B. R. Cho, H. M. Kim, A mitochondria-localized two-photon fluorescent probe for ratiometric imaging of hydrogen peroxide in live tissue, Chem. Commun., 48 (2012), pp. 3518-3520.

[11] R. X. Santos, S. C. Correia, X. L. Wang, G. Perry, M. A. Smith, P. I. Moreira, X. W. Zhu, A synergistic dysfunction of mitochondrial fission/fusion dynamics and mitophagy in Alzheimer's disease, J. Alzheimers Dis., 20 (2010), pp. 401-412.

[12] Y. Koide, Y. Urano, S. Kenmoku, H. Kojima, T. Nagano, Design and synthesis of fluorescent probes for selective detection of highly reactive oxygen species in mitochondria of living cells, J. Am. Chem. Soc., 129 (2007), pp. 10324-10325.

[13] C. S. Lim, G. Masanta, H. J. Kim, J. H. Han, H. M. Kim, B. R. Cho, Ratiometric detection of mitochondrial thiols with a two-photon fluorescent probe, J. Am. Chem. Soc., 133 (2011), pp. 11132-11135.

[14] A. Baracca, G. Sgarbi, G. Solaini, G. Lenaz, Rhodamine 123 as a probe of mitochondrial membrane potential: evaluation of proton flux through F_0 during ATP synthesis, Biochim. Biophys. Acta, Bioenerg, 1606 (2003), pp. 137-146.

[15] B. C. Dickinson, V. S. Lin, C. J. Chang, Preparation and use of MitoPY1 for imaging hydrogen peroxide in mitochondria of live cells, Nat. Protoc., 8 (2013), pp. 1249-1259.

[16] L. V. Johnson, M. L. Walsh, L. B. Chen, Localization of mitochondria in living cells with rhodamine 123, PNAS., 77 (1980), pp. 990-994.

[17] V. R. Fantin, P. Leder, F16, a mitochondriotoxic compound, triggers apoptosis or necrosis depending on the genetic background of the target carcinoma cell, Cancer Res., 64 (2004), pp. 329-336.

[18] H. He, D. W. Li, L. Y. Yang, L. Fu, X. J. Zhu, W. K. Wong, F. L. Jiang, Y. Liu, A novel bifunctional mitochondria-targeted anticancer agent with high selectivity for cancer cells, Sci. Rep., 5 (2015), pp. 13543.

[19] L. Fu, F. F. Tian, L. Lai, Y. Liu, P. D. Harvey, F. L. Jiang, A ratiometric

"two-in-one" fluorescent chemodosimeter for fluoride and hydrogen sulfide, Sens. Actuators, B., 193 (2014), pp. 701-707.

[20] L. Fu, F. F. Wang, T. Gao, R. Huang, H. He, F. L. Jiang and Y. Liu, Highly efficient fluorescent BODIPY dyes for reaction-based sensing of fluoride ions, Sens. Actuators, B., 216 (2015), pp. 558-562.

[21] T. Kowada, H. Maeda, K. Kikuchi, BODIPY-based probes for the fluorescence imaging of biomolecules in living cells, Chem. Soc. Rev., 44 (2015), pp. 4953-4972.

[22] A. T. Hoye, J. E. Davoren, P. Wipf, Targeting mitochondria, Acc. Chem. Res., 41 (2008), pp. 87-97.

[23] L. F. Yousif, K. M. Stewart, S. O. Kelley, Targeting Mitochondria with Organelle‐Specific Compounds: Strategies and Applications, ChemBioChem., 10 (2009), pp. 1939-1950.

[24] L. Fu, F. L. Jiang, D. Fortin, P. D. Harvey, Y. Liu, A reaction-based chromogenic and fluorescent chemodosimeter for fluoride anions, Chem. Commun., 47 (2011), pp. 5503-5505.

[25] H. B. Xiao, P. Li, W. Zhang, B. Tang, An ultrasensitive near-infrared ratiometric fluorescent probe for imaging mitochondrial polarity in live cells and in vivo, Chem. Sci., 7 (2016), pp. 1588-1593.

[26] N. Jiang, J. L. Fan, F. Xu, X. J. Peng, H. Y. Mu, J. Y. Wang, X. Q. Xiong, Ratiometric fluorescence imaging of cellular polarity: decrease in mitochondrial polarity in cancer cells, Angew. Chem. Int. Ed., 127 (2015), pp. 2540-2544.

[27] A. Ojida, T. Sakamoto, M. Inoue, S. Fujishima, G. Lippens, I. Hamachi, Fluorescent BODIPY-based Zn (II) complex as a molecular probe for selective detection of neurofibrillary tangles in the brains of Alzheimer's disease patients, J. Am. Chem. Soc., 131 (2009), pp. 6543-6548.

[28] M. Brellier, G. Duportail, R. Baati, Convenient synthesis of water-soluble nitrilotriacetic acid (NTA) BODIPY dyes, Tetrahedron Lett., 51 (2010), pp. 1269-1272.

[29] A. Romieu, C. Massif, S. Rihn, G. Ulrich, R. Ziessel, P. Y. Renard, The first comparative study of the ability of different hydrophilic groups to water-solubilise

fluorescent BODIPY dyes, New J. Chem., 37 (2013), pp. 1016-1027.

[30] S. L. Zhu, J. T. Zhang, J. Janjanam, G. Vegesna, F. T. Luo, A. Tiwari, H. Y. Liu, Highly water-soluble BODIPY-based fluorescent probes for sensitive fluorescent sensing of zinc (II), J. Mater. Chem. B., 1 (2013), pp. 1722-1728.

[31] T. Bura, R. Ziessel, A PEGylated colorimetric and turn-on fluorescent sensor based on BODIPY for Hg (II) detection in water, Org. Lett., 13 (2011),pp. 4279-4289.

[32] S. L. Zhu, N. Dorh, J. T. Zhang, G. Vegesna, H. H. Li, F. T. Luo, A. Tiwari, H. Y. Liu, Highly water-soluble neutral near-infrared emissive BODIPY polymeric dyes, J. Mater. Chem., 22 (2012), pp. 2781-2790.

[33] Giri K. Vegesna, Srinivas R. Sripathi, J. T. Zhang, S. L. Zhu, W. L. He, F. T. Luo, Wan Jin Jahng, Megan Frost, H. Y. Liu, ACS Appl. Mater. Interfaces. 5 (2013), pp. 4107–4112.

[34] C. Bernhard, C. Goze, Y. Rousselin, F. Denat, First bodipy–DOTA derivatives as probes for bimodal imaging, Chem. Commun., 46 (2010), pp. 8267-8269.

[35] S. L. Niu, C. Massif, G. Ulrich, R. Ziessel, P. Y. Renard, A. Romieu, Water-solubilisation and bio-conjugation of a red-emitting BODIPY marker, Org. Biomol. Chem., 9 (2011), pp. 66-69.

[36] R. F. Kubin, A. N. Fletcher, Fluorescence quantum yields of some rhodamine dyes, J. Lumin., 27 (1983), pp. 455-462.

[37] M. F. C. Abad, G. D. Benedetto, P. J. Magalhães, L. Filippin, T. Pozzan, Mitochondrial pH monitored by a new engineered green fluorescent protein mutant, J. Biol. Chem., 279 (2004), pp. 11521-11529.

[38] J. Llopis, J. M. McCaffery, A. Miyawaki, M. G. Farquhar, R. Y. Tsien, Measurement of cytosolic, mitochondrial, and Golgi pH in single living cells with green fluorescent proteins, PNAS., 95 (1998), pp. 6803-6808.

[39] M. H. Lee, N. Park, C. Yi, J. H. Han, J. H. Hong, K. P. Kim, D. H. Kang, J. L. Sessler, C. Kang, J. S. Kim, Mitochondria-immobilized pH-sensitive off–on fluorescent probe, J. Am. Chem. Soc., 136 (2014), pp. 14136-14142.

[40] C. W. T. Leung, Y. N. Hong, S. J. Chen, E. G. Zhao, J. W. Yip Lam, B. Z. Tang, A photostable AIE luminogen for specific mitochondrial imaging and tracking, J. Am.

Chem. Soc., 135 (2012), pp. 62-65.

[41] R. Huang, B. B. Wang, X. M. Si-Tu, T. Gao, F. F. Wang, H. He, X. Y. Fan, F. L. Jiang, Y. Liu, A lysosome-targeted fluorescent sensor for the detection of glutathione in cells with an extremely fast response, Chem. Commun., 52 (2016), pp. 11579-11582

[42] L. Zhang, W. W. Liu, X. H. Huang, G. X. Zhang, X. F. Wang, Z. Wang, D. Q. Zhang, X. Y. Jiang, Old is new again: a chemical probe for targeting mitochondria and monitoring mitochondrial membrane potential in cells, Analyst, 140 (2015), pp. 5849-5854.

[43] Q. F. Zhuang, H. Y. Jia, L. B. Du, Y. C. Li, Z. Chen, S. P. Huang, Y. Liu, Targeted surface-functionalized gold nanoclusters for mitochondrial imaging, Biosens. Bioelectron, 55 (2014), pp. 76-82.