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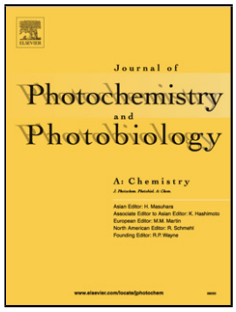

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Engineering a double-rotor-based fluorescent molecule to sensitively track mitochondrial viscosity in living cells and zebrafish with high signal-to-background ratio (S/B)

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Highlights

- An innovative design strategy has been proposed to construct viscosity-sensitive fluorescent probes (DRVPs) with superior sensitivity and high signal-to-background ratio (S/B) based on double-rotor molecular platform. DRVPs is more sensitive toward viscosity and performs higher S/B ratio comparing with the traditional single-rotor-based viscosity probes (SRVPs)
- To carry out a proof-of-concept project, the probe **DRVP** containing two rotors, quinolinium and carbazole moieties, was developed using a strong intramolecular charge transfer (ICT) platform. **DRVP** gave rise to an obvious fluorescence enhancement (51.8-fold) in 99% v/v glycerol solution.
- The cationic probe **DRVP** could clearly stain the mitochondria in HepG2 cells, and it can be successfully applied for the imaging of exogenous viscosity in living cells and zebrafish.

Abstract

An innovative design strategy has been proposed to construct viscosity-sensitive fluorescent probes with superior sensitivity and high signal-to-background ratio (S/B) based on double-rotor molecular platform. As a proof-of-concept, a double-rotor-based fluorescent molecule (**DRVP**) containing two rotors, quinolinium and carbazole moieties, was developed to track viscosity using a strong ICT platform. The cationic probe **DRVP** is able to visualize the mitochondrial viscosity variations in living cells and zebrafish.

Keywords

Fluorescent probe, High signal-to-background ratio, Viscosity, Mitochondria-targeted, Fluorescence imaging;

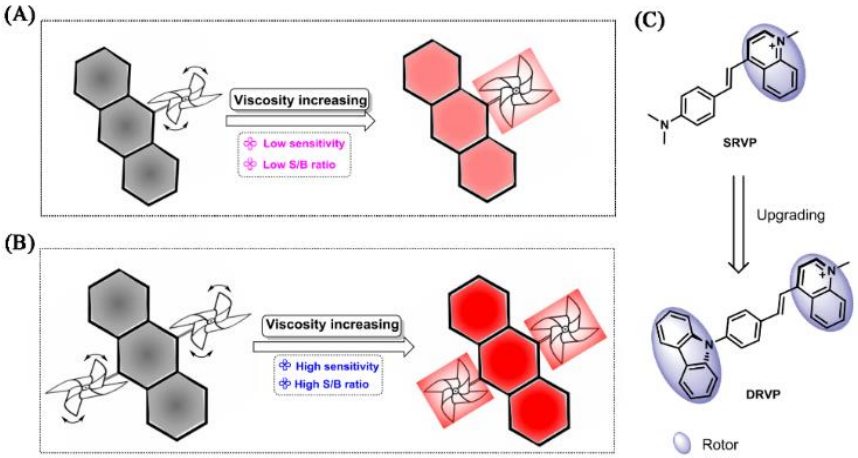
1. Introduction

Viscosity is an essential micro environment parameter in various biological systems, directly affecting transmission of eukaryotic cells chemical signals [1-5]. Changes of cell viscosity are related with some disease and dysfunction, such as hypertension, diabetes, cancers and so on [6-10]. Detection of intracellular viscosity remains one of the challenges in chemical biology. Mitochondria, as an important organelle, exist in most eukaryotic cells, which provide energy for the cells and are energy-generating compartments in cells [11-16]. Researches have shown that abnormal mitochondrial viscosity levels lead to the loss of mitochondrial activation, triggering numerous diseases including cerebral embolism, atherosclearosis, Alzheimer's disease (AD) and diabetes [17-28]. To this end, it is vital to develop effective strategy of monitoring the mitochondrial viscosity in biological systems for better understanding the mechanism of physiological and pathological functions in mitochondria.

Several analytical methods, such as chemiluminescence, capillary electrophoresis, spectrophotometry and electrochemical, have been developed to detect viscosity. However, the above methods fail to meet the measure of in situ viscosity in the biological systems [29-31]. Till now, fluorescence analysis technology based probes remains the mainstream tool for imaging of viscosity in biological and pathology due to its high sensitivity, real-time detection, fast response, low detection limit, and good biocompatibility [32-34]. Therefore, fluorescent probes with excellent response characteristics, particularly high sensitivity and S/B ratio, were proposed to be key for monitoring viscosity. It’s well known that molecular rotor is sensitive to viscosity variations. The free rotation of rotor moiety is one kind of non-radiation transition processes, which would relax the excitation energy and induce the fluorescence quenching of the molecular rotors. From this we speculated that the molecules containing multiple rotor groups would be of avail to achieve the low emission in the non-viscous media, and accordingly perform superior signal-to-background ratio (S/B) in the viscous situations. Although viscosity-sensitive fluorescent probes based on various molecular rotors have been developed, most of them only possess single rotor and sense viscosity with low signal-to-background ratio [35-41]. Dimer rotors have been reported, however, there is no evidence

that double-rotor-based fluorescent probe performs higher S/B ratio toward viscosity, comparing with single-rotor-based viscosity probes [42]. In this work, an innovative design strategy was proposed for constructing a fluorescent molecular containing two rotors with high signal-to-background ratio (S/B) toward viscosity. Notably, comparing with the traditional single-rotor-based viscosity probes (SRVPs) consisting of single rotor and chromophore core, the new type of double-rotor viscosity probes (DRVPs) perform the more advanced sensitivity and S/B through alleviation of adverse interference from the background emission signal (the emission from rotors in the non-viscous medium). In non-viscous situation, the low fluorescence of DRVPs should be attributed to the distortion of rotor moiety to form a non-planar platform with the inefficient conjugation effect. However, DRVPs show strong fluorescence in highly viscous systems because the rotation of rotor could be suppressed by the high viscosity media.

To perform a proof-of-concept project based on this new design strategy, we established a double-rotor-based fluorescent probe with superior S/B ratio for monitoring viscosity in biological systems. The quinolinium moiety and carbazole moiety were selected as two rotors, meanwhile serving as the electron-withdrawing group and electron-donating group, respectively, which were combined by the benzene ring building a strong intramolecular charge transfer (ICT) platform, as well as a double-rotor-based viscosity probe (**DRVP**, Scheme 1B). Compared with control fluorescent viscosity probe (**SRVP**) based on single-rotor molecular, the probe **DRVP** showed a more remarkable enhancement (51.8-fold) of the response signal induced by the increasing viscosity. In addition, the cationic skeleton of the probe **DRVP** should be an appropriate mitochondrial targeting group, which can promote the penetration of probe into the mitochondria through the plasma membrane. The desirable performances of turn-on response fluorescence signal were also observed in the confocal fluorescence images of living monensin/nystatin-mediated HepG2 cells and zebrafish. Thus, the probe **DRVP** is able to monitor mitochondrial viscosity variations with high sensitivity and S/B ratio in biological systems.



Scheme 1. The design strategy of double-rotor-based fluorescent probes with high sensitivity and S/B ratio toward viscosity. (A) The diagram and limitations of the traditional fluorescent viscosity probes (SRVPs) based on the molecular rotor containing single rotor group; (B) The diagram and advanced features of the new type of fluorescent viscosity probes (DRVPs) based on the molecular rotor containing dual rotor groups; (C) The chemical structures of the single-rotor-based viscosity probe (**SRVP**) and the double-rotors-based viscosity probe (**DRVP**) presented in this work.

2. Experimental

*2.1. Synthesis of compound **SRVP***

Compound **1** (30 mg, 0.2 mmol), compound **3** (57 mg, 0.2 mmol), were dissolved in ethanol (10 ml), piperidine (100 μL) and glacial acetic acid (100 μL) was added. Then, the mixture was refluxed at 80℃ for 12 hours. After the reaction, cooling and filtration, it was washed twice with ethanol to give a purple solid (67 mg, yield 80.5%), which was used in characterization and further reaction without purification. ^{1}H NMR (400 MHz, DMSO), δ (ppm): 3.074 (s, 6H), 4.447 (s, 3H), 6.815-6.837 (d, J = 4.4 Hz, 2H), 7.864-7.886 (d, J = 4.4 Hz, 2H), 7.960-8.035 (m, 2H), 8.168-8.230 (dd, J_1 = 3.4 Hz, J_2 = 12.4 Hz, 2H), 8.333-8.358 (m, 2H), 9.025-9.046 (d, J = 4.2 Hz, 1H), 9.114-9.131 (d, J = 3.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO): δ (ppm): 39.386, 39.595, 39.804, 40.012 40.219, 40.430, 40.638, 44.493, 112.368, 113.586, 114.415, 119.506, 123.527, 126.220, 126.813, 129.012, 131.251, 135.071, 139.251, 145.150, 147.241, 152.766, 153.628. HRMS (ESI) m/z calcd for $C_{20}H_{21}N_2^+$ $[M]^+$: 289.1699. Found 289.1696.

*2.2 Synthesis of the probe **DRVP***

Compound **3** (54.2 mg, 0.2 mmol), compound **6** (57 mg, 0.2 mmol), were dissolved in ethanol (10 ml), piperidine (100 μL) and glacial acetic acid (100 μL) was added. Then, the mixture was refluxed at 80℃ for 12 hours. After the reaction, the crude product was obtained by distillation under reduced pressure. The crude product was purified by column chromatography on silica gel using CH_2Cl_2/MeOH (v/v 15:1) as eluent, then the collection solution of compound **DRVP** was concentrated under reduced pressure to afford a red solid (82 mg, yield 76.2%). ^{1}H NMR (400 MHz, DMSO), δ (ppm): 4.992 (s, 3H), 7.319-7.353 (t, *J* = 6.8 Hz, 2H), 7.465-7.528 (q, *J* = 12.6 Hz, 4H), 7.826-7.847 (d, *J* = 4.2 Hz, 2H), 8.066-8.104 (t, *J* = 3.8 Hz 1H), 8.270-8.341 (m, 6H), 8.466-8.499 (m, 2H), 8.570-8.586 (d, *J* = 3.2 Hz, 1H), 9.106-9.127 (d, *J* = 4.2 Hz, 1H), 9.410-9.426 (d, *J* = 3.2 Hz, 1H); ^{13}C NMR (100 MHz, DMSO): δ (ppm): 39.391, 39.600, 39.809, 40.017, 40.226, 40.435, 40.643, 45.300, 110.257, 116.997, 119.892, 120.927, 121.094, 123.489, 126.895, 126.989, 127.299, 129.835, 131.042, 134.925, 135.525, 139.140, 139.233, 140.211, 142.233, 148.698, 152.871. HRMS (ESI) m/z calcd for $C_{30}H_{23}N_2^+$ $[M]^+$: 411.1856. Found 411.1858.

2.3. Fluorescence imaging in living cells

The monensin or nystatin (10 μM) was first added into the HepG2 cells in a glass bottom culture dishes (Nest) for 30 min, then they were washed by PBS for three times, after that, the probe **DRVP** (10 μM) was added into the HepG2 cells. The fluorescence emission of the probe was collected at TRICT channel (570 nm-620 nm), the excitation wavelength was 561 nm. monensin and nystatin could induce structural changes or swelling, leading to viscosity changes in the mitochondria.

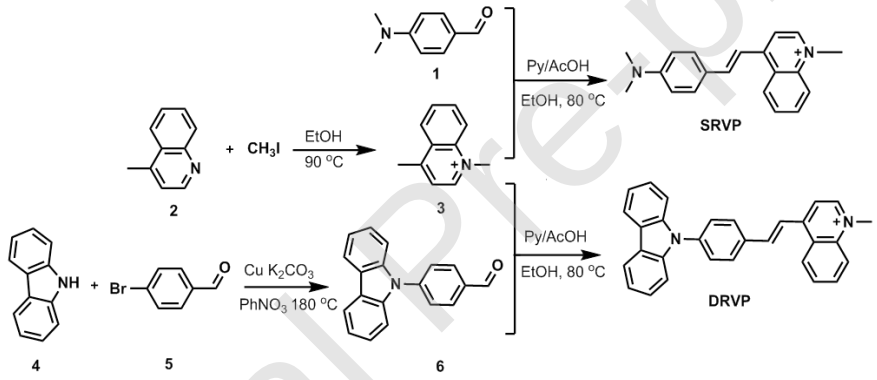
2.4. Fluorescence imaging in zebrafish

The 10 μM monensin or nystatin was added into the zebrafish for incubated for 30 min, followed by washing away gently. Then the probe **DRVP** (10 μM) was put into dishes for another 30 min. After that, the zebrafish was transferred into new glass bottom dishes for imaging. The fluorescence emission was collected at TRICT channel (570 nm-620 nm) upon excitation at 561 nm.

3 Results and discussion

*3.1. Design and synthesis of dyad **DRVP** and **SRVP**.*

In order to construct a double-rotor-based fluorescent probe with high S/B ratio, the quinolinium and carbazole moieties were selected as the two rotors, quinolinium as well as mitochondria-targeted group, binding these two rotors via the benzene ring to constitute the targeted double-rotor-based viscosity probe (**DRVP**). To prove the superior response properties of probe **DRVP** towards viscosity, we also synthesized a single-rotor-based viscosity probe (**SRVP**, Scheme 1C), which contains only one quinolinium rotor. The compound **SRVP** and the target probe **DRVP** was successfully prepared (Scheme 2). The compounds **3** and **6** were prepared by the previous methods [43-44]. The control probe **SRVP** and target probe **DRVP** were simply synthesized via the condensation reaction between the compound **3** and compound **1** or **6**, respectively. Characterization of all unknown samples was obtained by NMR spectroscopy and mass spectrometry.



Scheme 2. Synthesis route of the control probe **SRVP** and the probe **DRVP**.

*3.2. The optical fluorescence properties of **DRVP** and **SRVP** towards viscosity*

With the control probe **SRVP** and the target probe **DRVP** in the hands, the optical response capability of **SRVP** and **DRVP** to the viscosity was evaluated by emission spectra. Firstly, in order to test the influence of dimethylamino moiety rotation on emission, we selected a rigidfluorescent quinolone dye with a dimethylamino group (dimethylaminoquinoline-6-amine, **TQA** (Scheme S1)) for spectral verification. As the viscosity increases, the **TQA** showed a slight fluorescence enhancement (2.09-fold) in emission spectra (Fig S1), which should be contributed to the suppression of twisted intramolecular charge

transfer (TICT) effect due to the inhibiting rotation of dimethylamino group in viscous media. Thus the quinolinium moiety should play a primary role of rotation on compound **SRVP** rather than the dimethylamino group with small weight. Then the emission spectra of **SRVP** and **DRVP** were also measured in MeOH and glycerol. As shown in Fig. 1, both **SRVP** and **DRVP** were initially almost non-fluorescent in methanol, but their emission significantly enhanced with increase of the glycerol proportion in the methanol-glycerol mixture (Fig. 2 and S2). However, due to the much lower background fluorescence, probe **DRVP** gave rise to a more obvious fluorescence enhancement at 640 nm (51.8-fold) than that of the reference **SRVP** (34.7-fold) at 680 nm in 99% v/v glycerol solution. Meanwhile, 30 μM of probes **DRVP** and **SRVP** are capable for detecting the minimum 1.29 and 1.75 cp of viscosity, respectively, according to the linear relationship between viscosity values (η) of testing media and response fluorescence intensity (Fig. 1C and 1D). In addition, a good linear relationship between log I_{640} and log η (R^2=0.9919) was obtained by the Förster-Hoffmann equation (log$I$ = C + xlog η) [45], and the value of x (0.6154 and 0.5934), manifested that **DRVP** and **SRVP** might be used to quantitatively monitor viscosity changes *in vitro* (the inset in Fig. 1C and 1D).Collectively, those results indicated that **DRVP** is more sensitive toward viscosity and performs higher S/B ratio comparing with the control probe **SRVP**, hence the double-rotor-based viscosity probe (**DRVP**) is likely favourable in application of sensing viscosity.

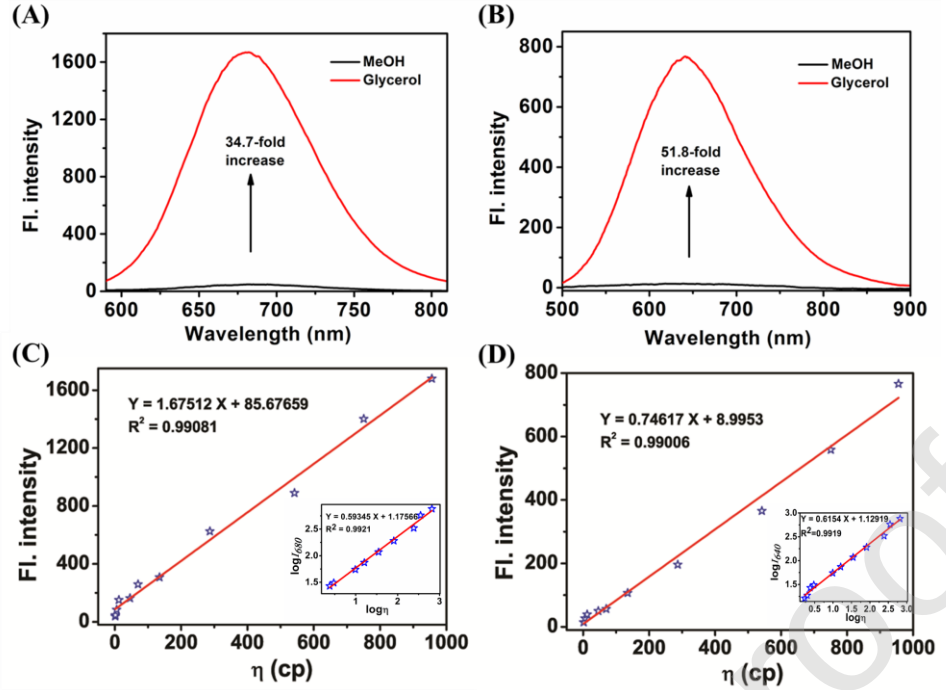


Fig. 1 (A) The emission spectra of **SRVP** (30 μM) in the MeOH (black) and MeOH-glycerol mixture (v/v 1/99, red) with excitation at 550 nm. (B) The emission spectra of **DRVP** (30 μM) in the MeOH (black) and glycerol (red) with excitation at 450 nm. The linear relationship between of fluorescence intensity and the viscosity values (η) for **SRVP** (C) and **DRVP** (D). Inset: the linear relationship between log I_{640} and log η for **SRVP** (R^2 = 0.9921, K = 059348) and **DRVP** (R^2 = 0.9919, K = 0.6154).

*3.3. Optical response of **DRVP** to viscosity*

After the initial photophysical characterisation for **DRVP** and **SRVP** towards viscosity, then we studied the response performance of probe **DRVP** (30 μM) to viscosity owing to its superior sensitivity and S/B ratio. As shown in Fig. 2A, **DRVP** displayed feeble emission at 640 nm, which steadily increased with the addition glycerol (the Φ_f of **DRVP** increased from 0.00048 to 0.01224). The result of viscosity-responsive signal would be ascribed to the prohibition of rotation in the more viscous solvents and the enhanced degree of conjugation. What's more, the greater enhancement (241.9-fold) of the fluorescence intensity was achieved in methanol castor oil mixture (Fig. S3). The fluorescence lifetime studies were also carried out in methanol and methanol/glycerol mixture, respectively. According to the fitting curves (Fig. S4), the fluorescence decay is double exponential. The fluorescence lifetime of

DRVP increased from 0.21 ns in absolute methanol to 1.51 ns in methanol/glycerol mixture solution using the formula ($\tau = \tau_1$*Rel$_1$% + τ_2*Rel$_2$%). These results indicated that the probe **DRVP** is a viscosity-sensitive probe. Besides, given the extremely complex intracellular environment, To study what extent influence on emission by solvent polarity, the fluorescence spectra of probe **DRVP** were tested in various solvents (DMF, DMSO, MeOH, acetone, THF, MeCN, DCM, $CHCl_3$, Toluene and ethylene glycol) with different polarity. As shown in Fig. S5, the probe exhibited weak fluorescence intensity in most non-viscous solvents except $CHCl_3$, while it showed strong fluorescence emission in viscous solvent, glycerol. Though the emission wavelengths were quite varied, the emission were very weak in almost all nonviscouse solvents but glycerol. The results indicated that the emission intensity of **DRVP** is mainly effected by the solvent viscosity rather than solvent polarity.

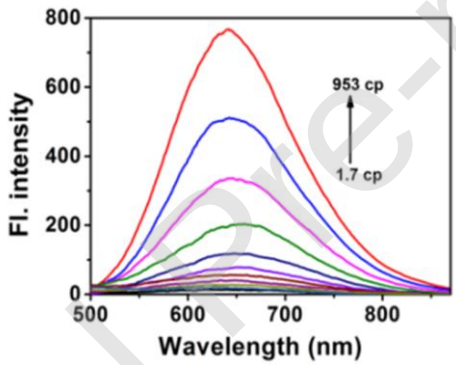


Fig. 2 (A) The fluorescence emission spectra of **DRVP** (30 μM) in methanol-glycerol systems at various ratios. λ_{ex} =450 nm.

The selectivity of probe is used as an extremely significant parameter due to the fact that cell is a complex multicomponent system [46]. Therefore, the fluorescence spectra of **DRVP** were observed in the presence of competing biomolecules. The selectivity of the probe **DRVP** was further assessed by co-existence with various cations (Al^{3+}, Ba^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Mg^{2+}, Na^{+}, Zn^{2+}), anions (NO_2^-, NO_3^-, $S_2O_3^-$, SO_3^{2-}, CNS^-), sulphur-containing (Cys, GSH, Hcy), reactive oxygen species (H_2O_2, HClO), and amino acids species (alanine, arginine, histidine, isoleucine, glutamate). As shown Fig.3A, the probe **DRVP** treated with these biological species caused tiny signal fluctuations, only more viscous solvents would cause significant fluorescence enhancement at 640 nm. These results indicated that the probe **DRVP**

has excellent selectivity for glycerol. What's more, **DRVP** displayed almost non-fluorescence changes at 640 nm under various pH values (Fig. 3B).With the aim of gaining further insight into the photostability of **DRVP**, It was observed that the fluorescence intensity has tiny change of at 640 nm (Fig. S6). As a result, the probe **DRVP** might be stable in the biological situations and has the relative good photostability.

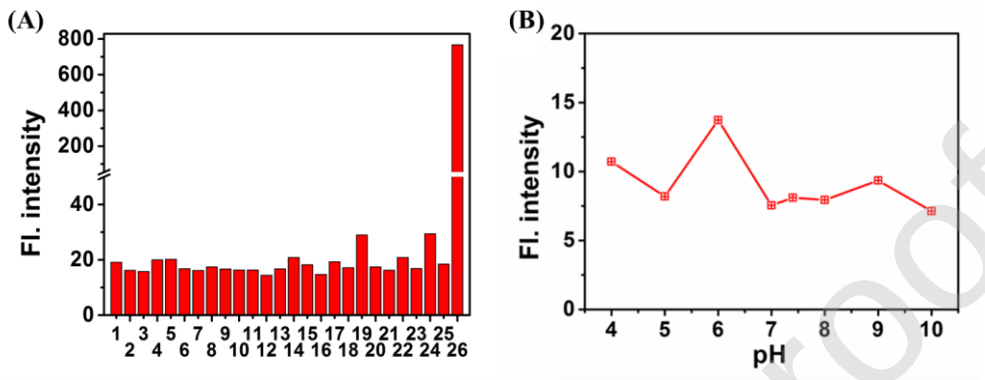


Fig. 3 (A). The fluorescence intensity of **DRVP** (30 μM) at 640 nm in MeOH solution. 1: Blank; 2: Al^{3+}, 3:Ba^{2+}; 4:Ca^{2+}; 5: Co^{2+}; 6: Cu^{2+}; 7: Fe^{3+}; 8: Mg^{2+}; 9: Na^{+}; 10: Zn^{2+}; 11: NO_2^-; 12: NO_3^-; 13: $S_2O_3^-$; 14: SO_3^{2-}; 15: CNS^-; 16: Cys; 17: GSH; 18: Hcy; 19: H_2O_2; 20, HClO; 21: Ala; 22: Arg; 23: His; 24: Ile; 25: Gln; 26: glycerol; (B) The effect of pH on the fluorescence intensity of **DRVP** (30 μM) at 640 nm in PBS buffer (containing 50% MeOH). The fluorescence intensity was measured at λ_{ex} = 450 nm with both excitation and emission slit widths of 5 nm, and 700 V PMT voltage.

3.4. Mitochondrial colocalization experiment

The co-localization experiment was performed, in consideration of the possibility that the probe **DRVP** has excellent membrane permeability and specifically enrich in mitochondria. Prior to this, the cytotoxicity of probe was evaluated by MTT assay because cytotoxicity is a momentous parameter for fluorescent probe. The cell viability is above 75% when incubated with **DRVP** (0, 5, 10, 25, or 50 μM) for 24 h, demonstrating that the influence of **DRVP** on cell viability could be omitted and used for cell imaging (Fig. S7) [47]. Then, the cell co-localization experiment of **DRVP** was confirmed by Mito-Tracker Green (MTG, commercial mitochondrial dye), HepG2 cells were incubated with **DRVP** (10 μM) for 30 minutes, and then cultured with 2.5 μM Mito-Tracker Green for 5 minutes. The imaging results indicated that the

combined images of the Mito-Tracker Green channel and the cell channel could overlap very well. The intensity profile variations in the green and red channels exhibited a tendency toward synchrony. What's more, a high correlation of 0.86 was calculated by the Pearson correlation coefficients (Fig. 4). These experimental results indicated that **DRVP** with high S/B ratio could be used as a targeting probe for specific enrichment in mitochondria.

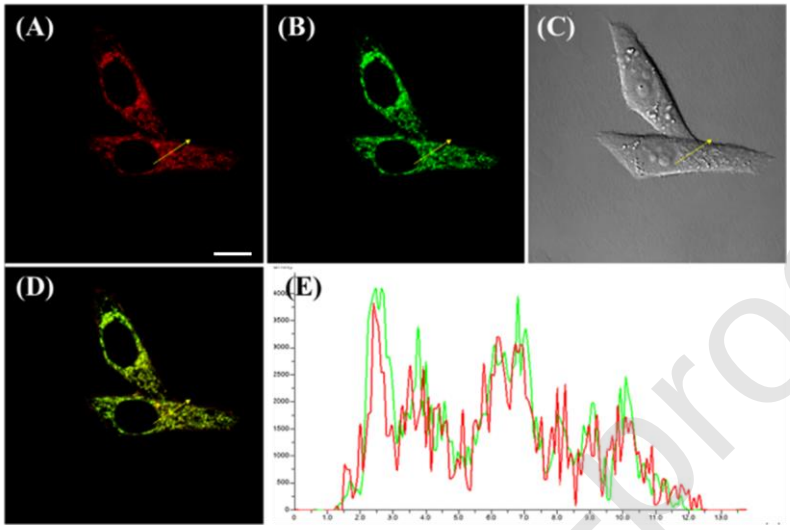


Fig. 4 The confocal fluorescence images of HepG2 cells co-incubated with with **DRVP** (10 μM) and Mito-Tracker Green (2.5 μM) for 5 min. (A) Red channel image by excitation at 561 nm; (B) Green channel image by excitation at 488 nm; (C) Bright field image; (D) Merged images of (A) and (B); (E) Intensity profile of the ROI indicated by arrow in (D). Scale bar: 25 μm.

3.5. Living cell imaging

Subsequently, we further demonstrate the ability of the probe **DRVP** for cell imaging of viscosity variations. External stimuli (monensin and nystatin) are used to induce mitochondrial disease by stimulating its viscosity increase. When HepG2 cells were incubated with **DRVP** (10 μM) for 30 minutes, the probe **DRVP** was observed to weak red fluorescence in the TRITC channel (Fig. 5A). In contrast, when the cells were pre-treated with nystatin or monensin for 30 minutes, and then stained with **DRVP** for 30 minutes, the red fluorescence was significantly enhanced in the TRITC channel (Fig. 5B-C). In addition, the cell imaging experiments using compound **SRVP** were also

performed as a comparison. As shown in S8, probe **SRVP** exhibited 3.0/2.4-fold induced by monensin/nystatin stimulation in living cells. In contrast, probe **DRVP** showed 5.9/4.0-fold and 4.3/3.5-fold increase induced by the same drugs stimulation in living cells (Fig. 5). Overall, compared with **SRVP**, probe **DRVP** had the advantages of high S/B ratio and high sensitivity in living cells.

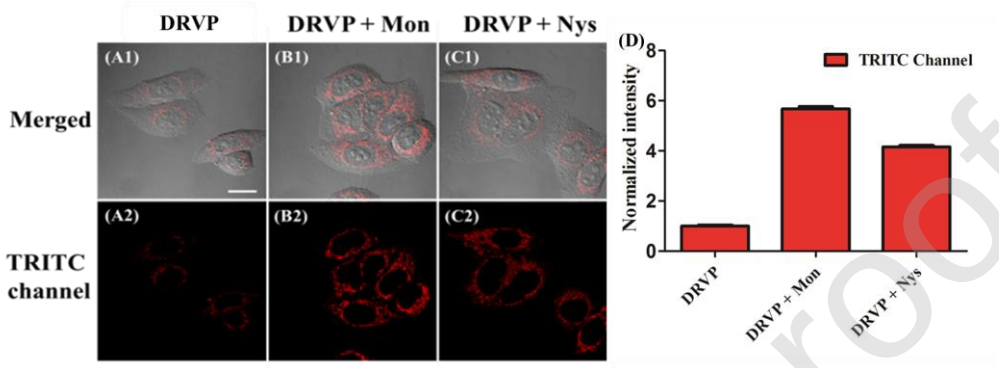


Fig. 5 Confocal fluorescence images of the probe **DRVP** responding to exogenous viscosity in living cells. (A) HepG2 cells were incubated with only probe **DRVP** (10 μM) for 30 min. (B-C) HepG2 cells were pretreated with (B) monensin (10 μM) or (C) nystatin (10 μM) for 30 min, followed by incubation of probe **DRVP** (10 μM) for another 30 min. First line: merged images of bright filed and TRITC channels; second line: TRITC channel (570-620 nm); Scale bar: 25 μm. (D) Normalized intensity in TRITC channel in Fig (A2-C2).

3.6. Zebrafish fluorescence imaging

Furthermore, we also explored the potential application of the viscosity variations in living zebrafish. As shown in Fig 6, the zebrafish incubated with **DRVP** (10 μM) showed weak red fluorescence. However, the zebrafish successively treated with 10 μM of monensin or nystatin and probe **DRVP** (10 μM) showed a significant increase of fluorescence intensity. In addition, the zebrafish imaging experiments using compound **SRVP** were also performed as a comparison. As shown in Fig. S9, probe **SRVP** exhibited 2.9/2.3-fold increase of fluorescence induced by monensin/nystatin stimulation in living zebrafish, respectively. In contrast, probe **DRVP** showed 4.3/3.5-fold increase induced by the same drugs stimulation in living cells and zebrafish,

respectively. The higher S/B ratio detection by probe **DRVP** should be contributed to the lower background fluorescence produced by **DRVP** in the relatively low viscous environment. Thus, compared with **SRVP**, probe **DRVP** had the advantages of high S/B ratio and high sensitivity in biological systems.

What's more, adding nystatin or monensin may changes the combining ability of **DRVP** to mitochondria, leading to a higher fluorescence intensity in living cells and zebrafish. To explain what the primary factor for leading to the fluorescence increase, we selected quinolin fluorophore (N, N, 2-trimethylquinolin-6-amine, **TQA**) and carbazole fluorophore (3-aldehyde-9-ethylcarbazole, **AEC**) (Scheme S1), two core components being consisted of probe **DRVP**, for cell and zebrafish imaging at the same operating conditions. As expounded in Fig. S10 and S11, after stimulation of whether nystatin or monensin, the fluorescence intensity of the cells incubated with compound **TQA** or **AEC** hardly changed. However, after incubating with stimulus, the fluorescence intensity of the cells treated with probe **DRVP** in the TRITC channel significantly enhanced (Fig. 5). The similar change phenomena of fluorescence induced by nystatin or monensin were also observed in zebrafish (Fig. S12-13). Thus, the increasing viscosity of mitochondria should the primary factor rather than increasing probe concentration for leading to the fluorescence increase.

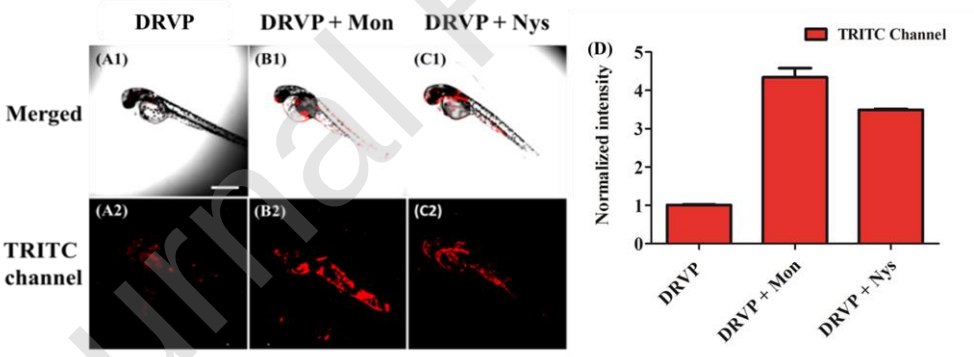


Fig. 6 Confocal fluorescence images of viscosity in living zebrafish. (A) Zebrafish was incubated with probe **DRVP** (10 μM) for 30 min. (B-C) zebrafish were pretreated with (B) monensin (10 μM) or (C) nystatin (10 μM) for 30 min, then incubated with probe **DRVP** (10 μM) for another 30 min. First line: merged images of bright filed and TRITC channels; second line: TRITC channel (570-620 nm); Scale bar: 250 μm. (D) Normalized intensity in TRITC channel in Fig (A2-C2).

4. Conclusions

In summary, we have proposed a new design concept to construct viscosity-sensitive fluorescent probes based on double-rotor molecular platform for sensing viscosity with excellent sensitivity and high signal-to-background ratio (S/B) in the complex biological system. To execute a proof-of-concept project, the probe **DRVP** containing two rotors, quinolinium and carbazole moieties, was developed using a strong ICT platform. It performs initially low background signal in methanol solution with low viscosity, while achieves a significant enhancement (51.8-fold) of fluorescence in viscous glycerol media. What's more, the cationic probe **DRVP** can specifically accumulate in mitochondria, and it has been demonstrated to be able to serve as a powerful tool for monitoring viscosity in living cells and zebrafish. We anticipate that the design strategy proposed in this work would accelerate the development of a diversity of fluorescent viscosity probes with superior S/B ratio and sensitivity, providing promising prospects for the future of biological and clinical research.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

W. Lin directed the research. Y. Yang and L. He designed and performed the experiments, analyzed the data, and wrote the paper. All the authors discussed the results and commented on the manuscript.

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