Supporting Information for

TICT mechanism-based viscosity fluorescent probe for monitoring inflammatory cell viscosity

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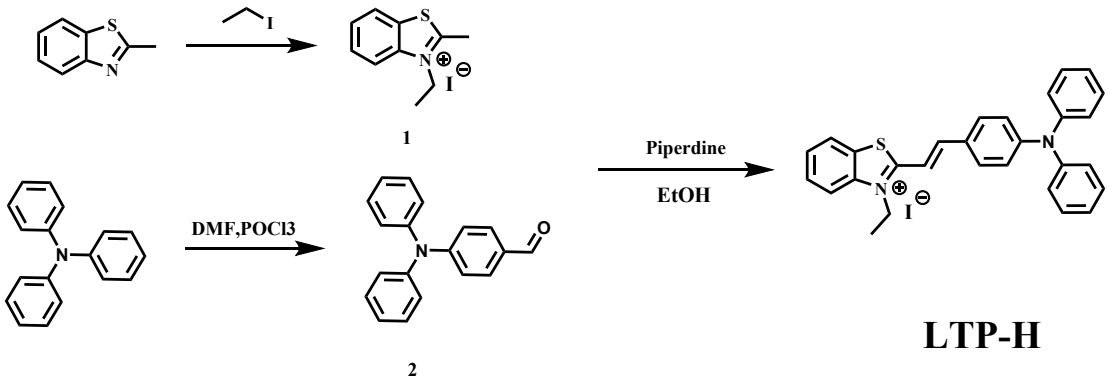
Contens

1. Materials and instruments

All reagents and materials were purchased from commercial companies and used without further purification unless otherwise stated. All aqueous solutions were prepared with ultra-pure water obtained from a Milli-Q water purification system (18.2 MΩ cm).

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance III HD 500 MHz NMR spectrometer (Germany). High resolution mass spectrometric (HRMS) analyses were measured on Aglient 6550 Q-TOF. The absorbance was recorded by ultraviolet-visible absorption spectrometry (UV-2700, Shimadzu) or microplate reader (TransGen Biotechnology). TLC analyses were carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both were purchased from the Qingdao Ocean Chemicals. Cells were photographed under the microscope (RVL-100-G, USA Discover-Echo, 60×). The fluorescence imaging of cells was performed with a Leica TCS SP8 CARS confocal microscope.

2. Synthesis of compound LTP-H



Scheme S1 Synthesis diagram of probe **LTP-H**

2.1 Synthesis of compound 1

Weigh (2 mL, 5.8 mmol) of 2-methylbenzothiazole and pour it into a 25 mL round bottom flask. Then add (2 mL, 25 mmol) of iodoethane and heat it in a water bath to around 85 ℃ for 18 h. The color of the solution gradually changes from yellow to black. After the reaction is complete, a solid is precipitated, filtered and washed with petroleum ether, and dried in a vacuum drying oven to obtain purple red block solid **compound 1**.Yield: 0.82 g (72.5%).

2.2 Synthesis of compound 2

Slowly add $POCl_3$ (8 mL, 2 eq) dropwise to a dry, anhydrous, and oxygen free N, N-dimethylformamide (4 mL, 1 eq) three necked flask in an ice water bath. After 30 min of reaction, weigh triphenylamine (1.50 g, 1.1 eq) and dissolve it in 2 mL of anhydrous N, N-dimethylformamide solution. Slowly inject the mixture into the three necked flask under nitrogen protection. After completion, transfer to a 60 ℃ oil bath for 8 h of reaction. After the reaction is complete, pour sufficient ice water to quench the excess phosphorus trichloride, and add solid sodium carbonate to adjust the pH of the solution to 7. Subsequently, the organic phase was extracted with dichloromethane and saturated sodium chloride aqueous solution. After drying with anhydrous sodium sulfate and rotary evaporation, a yellow crude product was obtained. The eluent was PE:EA=50:1 (v/v), and a light yellow solid **compound 2** was obtained.Yield: 1.42 g (85%).

2.3 Synthesis of compound **LTP-H**

Dissolve compound 1 (610.00 mg, 2.00 mmol) in anhydrous ethanol (6 mL) and stir at 80 ℃ for 1 h. Add dissolved compound 2 (364.00 mg, 1.33 mmol) and catalyst piperidine (100 μL), raise the temperature to 85 °C, condense and reflux under nitrogen protection for 12 h, and monitor the reaction by TLC. The crude product obtained by vacuum concentration at the end of the reaction was purified by column chromatography (DCM: MeOH=20:1,v/v), and the deep red solid compound **LTP-H** was obtained by recrystallization with ethanol.Yield: 447.00 mg (60%). 1H NMR (600

MHz, Chloroform-d) δ 8.79 (d, J = 4.4 Hz, 1H), 8.12 (s, 1H), 8.05 (d, J = 8.1 Hz, 1H), 8.01 (d, J = 8.3 Hz, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.72 (t, J = 6.9 Hz, 1H), 7.58 (d, J = 7.0 Hz, 1H), 7.55 (d, J = 8.3 Hz, 1H), 7.51 (d, J = 8.6 Hz, 2H), 7.47 (t, J = 8.2 Hz, 1H), 7.31 – 7.27 (m, 3H), 7.16 (dd, J = 15.2, 8.0 Hz, 4H), 7.08 – 7.03 (m, 2H), 6.62 (d, J = 8.2 Hz, 1H), 2.73 (s, 2H), 1.42 (s, 3H) .13C NMR (151 MHz, CDCl3) δ 190.437, 152.04, 149.15, 145.85, 139.81, 131.43, 130.48, 130.17, 127.90, 127.40, 126.80, 126.65, 125.99, 124.75, 121.66, 121.18, 112.20, 43.07, 13.51.HRMS (ESI): calcd. for $C29H25N2S^+$, $[M]^+$, m/z, 433.1738, found: 433.1739.

3. DFT calculations

Quantum chemical calculations based on density-functional theory (DFT) and time-varying density-functional theory (TD-DFT) were performed using the software Gaussian 09 and the B3LYP/6-31G(d,p) basis set. The initial geometry of the probe **LTP-H** was generated using the software Gaussian View.

4. Spectral test method

4.1 Probe test sample configuration

2.00 mg of Probe **LTP-H** solid was weighed and 3.57 mL of dimethylsulfoxide (DMSO) was added to make a 1.0 mM master mix, and all spectroscopic probes were at 10 μM. The concentration of all the spectroscopic probes was 10 μM. 20 μL of the probe master mix was added to 2.0 mL of a different solvent system to obtain a 10 μM dilution of Probe **LTP-H**.

4.2 Selective analyte configuration

A Stock solution with a concentration of 10 mM was prepared with ultrapure water, which included: Fe^{2+}; Fe^{3+}; Co^{2+} ; Hg^{+}; Ca^{2+}; Mg^{2+}; F^{-}; SCN^{-}; $H_2PO_4^{-}$; SO_4^{2-}; CO_3^{2-}; $ONOO^{-}$; HClO; HSO_3^{-}; $S_2O_3^{2-}$; Hcy; Ser ; the above mentioned. The test concentration of the selective analyte was 100 μM. 20 μL of the mother liquor was pipetted into 2 mL of the test system to obtain a 100 μM dilution of the selective analyte.

4.3 Preparation of PBS buffer solution

The procured analytical-grade phosphate buffer formulation powder was dissolved in ultrapure water, made up to the mark in a 2 L volumetric flask, the pH of the solution was recorded as 7.55, and autoclaved for use.

4.4 pH buffer system solution configuration

A pH (2.0-12.0) gradient system was constructed by microtitration of 0.1 M HCl/NaOH using PBS as the base buffer, which was confirmed by a pH meter calibration before the experiment.

5. Culture and preparation of HepG2 cells

HepG2 cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO2 and 95% air at 37 °C. Before the experiments, the HepG2 cells in 35-mm glass-bottomed dishes were cultured to a density of 2×105 cells per dish. Incubate the cells for 24 h. Cells will attach to the glass surface during this time.

6. Cytotoxicity assays

HepG2 Cells were inoculated into 96-well plates, and probe **LTP-H** (95% DMEM and 5% DMSO) of 0, 5, 10, 15, 20, 25, 30,40 and 50 μM (final concentration) were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 h. Then cells were washed with PBS buffer and DMEM medium was added. Next, MTT (10 μL, 5 mg/mL) was injected into each well and incubated for 4 h. Treatment with sodium dodecyl sulfate solution (100 μL) in H_2O-DMF mixture produced purple methyl. The viability of cells was determined by assuming that the viability of cells without **LTP-H** was 100%.

$$\text{Cell viability} = \frac{\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}}{\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}} \times 100\%$$

The experimental parameters were defined as follows: ODsample is the absorbance value of the experimental group containing the gradient concentration of **LTP-H**, ODcontrol is the absorbance value of the cell-only control group, and ODblank is the absorbance value of the medium blank group.

7. Cell imaging experiment

7.1 OA induced inflammatory imaging of HepG-2 cells

Prior to imaging, 1 mL of cells were seeded at a density of 1 × 105 /mL in a glass-bottomed petri dish (Nest). Cells were placed on glass coverslips and allowed to adhere for 24 hours. Imaging experiments were performed when the cells reached approximately 70% fusion. Oleic acid-induced cell imaging: in the first group, cells were incubated with PBS for 3 hours, then 10 μM probe **LTP-H** was added for 30 minutes; in the second group, cells were incubated with DMEM for 12 hours, then 10 μM probe **LTP-H** was added for 30 minutes; in the last group, cells were incubated with 2, 4, 6, and 8 μg/mL oleic acid for 1 hour, then 10 μM probe **LTP-H** was added for 30 minutes. The last group, cells were incubated with 2, 4, 6 and 8 μg/mL oleic acid for 1 hour, then 10 μM probe **LTP-H** was added and incubated for 30 minutes for confocal imaging. The cells were rinsed three times with PBS (10 mM, pH 7.4) before each imaging. λex = 570 nm, λem = 590-700 nm.

7.2 LPS induced inflammatory imaging of HepG-2 cells

LPS (lipopolysaccharide)-induced cell imaging: the first group was incubated with 10 μM probe **LTP-H** for 30 min; the second group of cells was incubated with 10 μg/mL LPS for 1 h, and 10 μM probe **LTP-H** for 30 min; the third group of cells was incubated with 20 μg/mL LPS for 1 h, and 10 μM probe **LTP-H** was incubated for 30 min and then confocal imaging was performed.The cells were rinsed three times with PBS (10 mM, pH 7.4) before each imaging. λex = 570 nm, λem = 590-700 nm.

8. Lysosomal co-localisation experiment:

Cells were incubated with probe **LTP-H** (10 μM) for 30 min at 37 °C in an

incubator with 95% air and 5% CO_2. HepG-2 cells were then incubated with Lyso-Tracker Green (10 μM) for 30 minutes. The medium was then removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) and confocal imaging was performed to analyse the lysosomal localisation ability of the probe **LTP-H** using Pearson's coefficient. (red channel: λex = 590 nm, λem = 560-700 nm; green channel: λex = 488 nm, λem = 500-550 nm).

9. Zebrafish imaging experiment

Wild-type zebrafish were purchased from Nanjing Eze Runjia Co. In accordance with the Chinese Law on the Use of Laboratory Animals, all procedures in this study were approved by the Animal Ethics Experiment Committee of Guangxi University.

For fluorescence imaging experiments, 3-day-old zebrafish were transferred into 30 mm glass Petri dishes using disposable sterilised droppers. The zebrafish were divided into control and experimental groups; the control group was incubated with the probe **LTP-H** (10 μM) for 30 min,followed by three PBS rinses prior to confocal imaging. In the experimental group, zebrafish were treated with 10 μM lipopolysaccharide, 10 μM monensin, 10 μM nystatin and 10 μM rapamycin in a Petri dish for 30 min, subsequently incubated with probe **LTP-H** for 30 min, and then transferred to a new glass dish for imaging. Before each imaging session, zebrafish were rinsed three times with PBS (10 mM, pH 7.4), fixed with 1% agarose gel,and mounted on agarose with a small volume of medium for confocal imaging (λex= 570 nm, λem = 590-700 nm).

10. Calculation of fluorescence quantum yield

The fluorescence quantum yield was calculated as follows:

$$\Phi_s = \Phi_r \left(\frac{n_s}{n_r}\right)^2 \left(\frac{A_r}{A_s}\right)\left(\frac{F_s}{F_r}\right)$$

The subscript r represents the reference compound (rhodamine B in ethanol Φs = 0.68 , λex = 570 nm), and the subscript s represents the probe and its controller. Φ is the fluorescence quantum yield, A is the absorbance of UV absorption, F is the area of the fluorescence emission peak, and n is the refractive index of the solution.

11. Calculation of detection limits

The limit of detection (LOD) is the smallest concentration or amount of the substance to be tested that can be detected with appropriate confidence, and the LOD calculation for a logarithmically fitted curve is calculated by backpropagation using the following formula:

$$\eta_{LOD} = 10^{\left(\frac{Log(\mu_{blank}+3\sigma_{blank})-b}{k}\right)}$$

Where μ_{blank} is the mean value of fluorescence intensity of the blank group (pure methanol), σ_{blank} is the standard deviation of the fluorescence intensity value of the blank group (pure methanol), k is the slope of the logarithmic linear fit curve of the fluorescence intensity versus the viscosity (logY=klogη+b),b is the intercept.

The specific calculation is as follows: based on the fluorescence intensity values of three blank samples, calculate the mean value (μ) and standard deviation (σ) respectively, subsequently obtain the detection limit signal value YLOD (μ+3σ), then substitute into the logarithmic linear fitting curve of fluorescence intensity versus viscosity and perform the antilogarithm operation to determine the probe's limit of detection (LOD).The detection limit of the probe **LTP-H** in this paper was 1.48 cP.

Supplementary Figures

Table 1 Photo-physical data of the probe **LTP-H** in different solvent systems

Solvents	ET (30)	λabs/nm	λem/nm	ε/L mol^{-1} cm^{-1}	Stokes shift/nm	ΦF (%)
Glycerol	57.2	570	650	67400	80	61.50
DMSO	45.1	572	650	63500	78	8.30
EtOH	51.9	567	649	54300	82	11.90
CH_3CN	45.8	562	650	27000	88	21.70
MeOH	55.5	570	650	33400	80	2.01
THF	37.4	575	650	58000	75	13.40
DMF	43.8	564	650	51200	86	10.60
1,4-Dioxane	36.0	560	649	22000	89	15.40

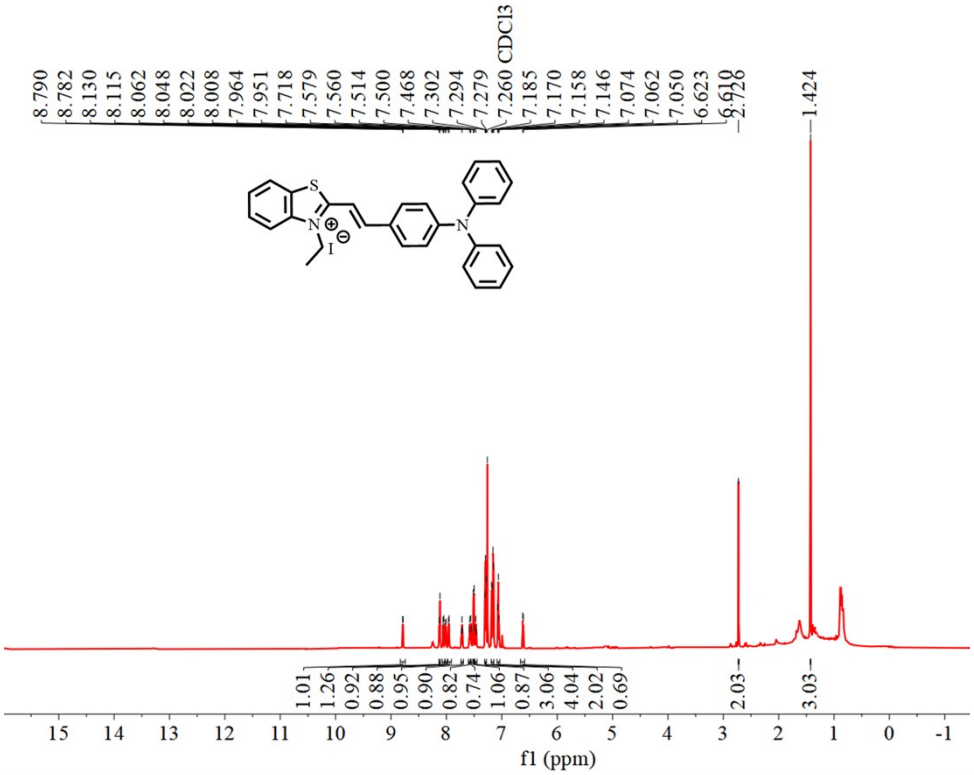


Fig. S1 The 1H NMR spectrum of probe **LTP-H**.

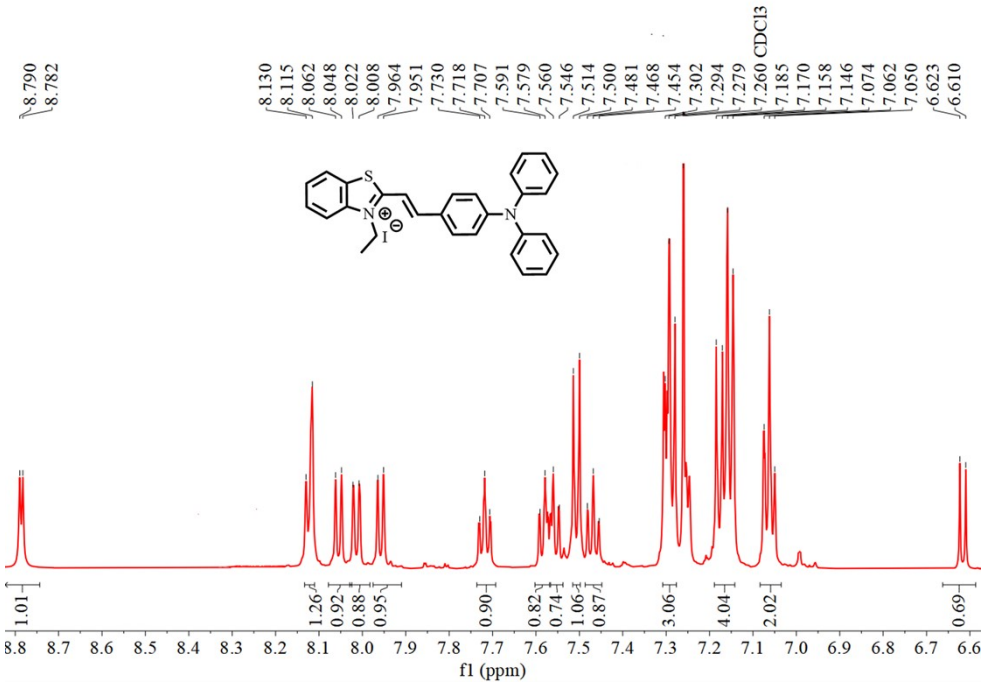


Fig. S2 Expanded 1H NMR spectrum of probe **LTP-H**.

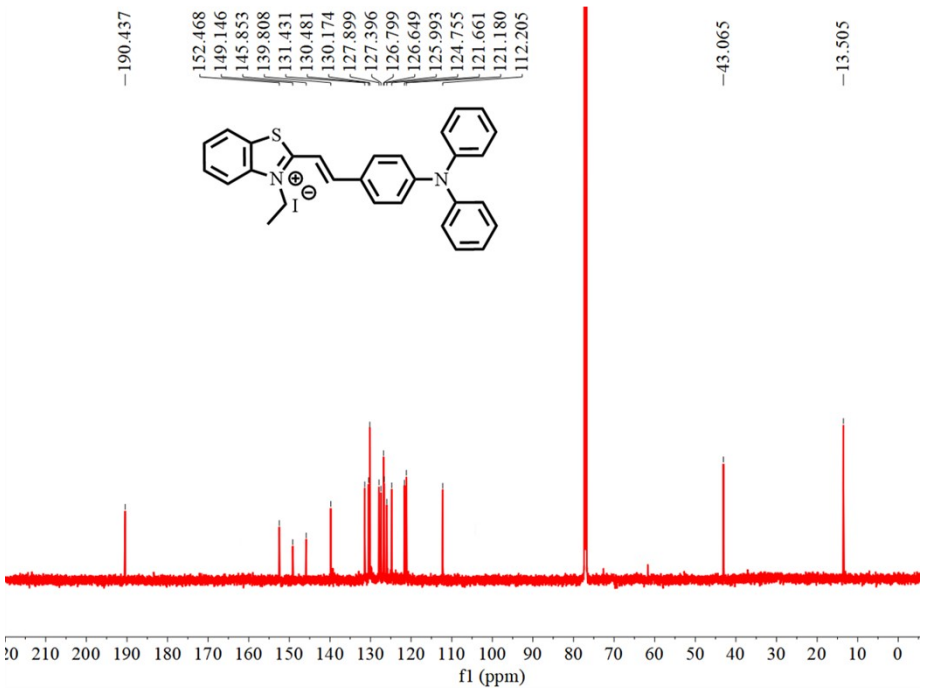


Fig. S3 The 13C NMR spectrum of probe **LTP-H**.

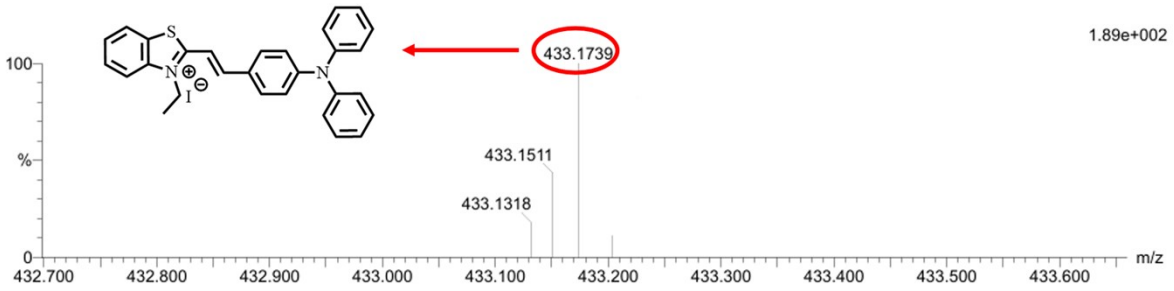
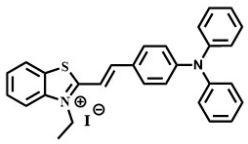
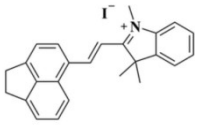
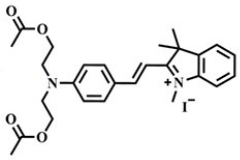
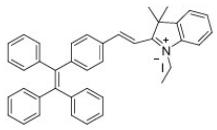
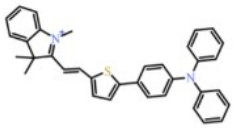
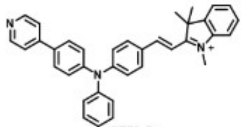
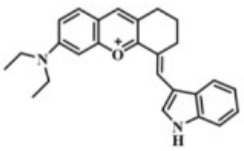
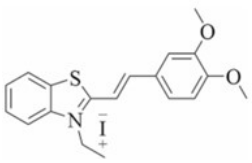
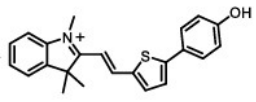


Fig. S4 HRMS spectrum of probe **LTP-H**.

Table 2 Viscosity of Glycerol and Methanol mixed at different volume ratios

Glycerol	Methanol	Viscosity / CP
99%	0	938.0
90%	10%	313.27
80%	20%	150.11
70%	30%	75.34
60%	40%	35.21
50%	50%	24.64
40%	60%	15.36
30%	70%	8.97
20%	80%	5.26
10%	90%	3.23
0	99%	1.30

Table 3 Comparison of this work with existing works

Probe	λex/λem(nm)	Stokes shift	viscosity response ratio	Cytotoxicity survival（%）	Bioimaging Applications	Ref.
	570/650	80	93	＞87	Cells Zebrafish	This work
	460/590	130	18	＞82	Cells Zebrafish	1
	515/590	75	23	＞80	Cells Tissue	2
	460/650	190	83	＞80	Cells	3
	600/750	150	65	＞81	Cells	4
	535/650	115	43	＞80	Cells	5
	648/705	57	17.5	＞95	Cells	6
	425/528	103	20	＞95	Cells	7
	510/628	118	16	＞90	Cells Zebrafish Mice	8

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