Supporting Information

A novel carbazolyl GFP chromophore analogue: Synthesis strategy and acidic pH-activatable lysosomal probe for tracing endogenous viscosity changes

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The Förrster–Hoffmann equations[1]

The Förrster–Hoffmann equations were utilized to correlate the relationship between the fluorescence emission intensity of Lys-CzFP and the value of solvent viscosity.

$\log FI = C + x \log \eta$

$\log \tau = C + x \log \eta$

Where η is the value of viscosity, FI is the emission intensity, τ is the fluorescence lifetime, C is a constant, and x represents the sensitivity of the fluorescent probe to viscosity.

Fluorescence quantum yields measurements[2]

The relative fluorescence quantum yields were determined with fluorescein in 0.1 M NaOH ($\phi_F = 0.95$) as a reference standard and calculated by the following equation:

$\phi_x = \phi_s(F_x/F_s)(A_s/A_x)(\lambda_{exs}/\lambda_{exx})(n_x/n_s)^2$

Where ϕ_F stands for quantum yield; F represents the integrated area under the appropriate emission spectrum; A stands for absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; n is the refractive index of the solution; and the subscripts x and s refer to test sample and reference substance, respectively

Cell culture.

MTT cytotoxicity assay[3]

Cytotoxicity of the probe Lys-CzFP was carried out by a standard MTT assay. The logarithmic phase of Bel-7402 cells was seeded into a 96-well cell culture plate (1×10^4 cells per well) and incubated under 95% air and 5% CO_2 at 37°C for 24 h. Next, the cells were incubated with different concentrations (0 mM, 5 mM, 10 mM, 15 mM, 20 mM, and 25 mM) of the probe Lys-CzFP for another 24 h. Then, the cells were incubated with 5 mg · mL^{-1} MTT for 4 h at 37°C under 95% air and 5% CO_2. After the supernatant was finally discarded, 100 mL DMSO was

added to each well, and oscillation was conducted for 5 minutes. The absorbance of each well of the 96-well plate was then assessed at 570 nm on a Benchmark Plus plate reader. Then, each cytotoxicity experiment was repeated three times by the standard method. The toxicity of the probe molecule Lys-CzFP was assessed by cell viability. The viability of the cells was calculated according to the following equation: Cell viability (%) = Experimental value OD570 / Control value OD570 × 100%, where OD570 is the absorbance measured at 570 nm.

Lysosomal co-location experiment[4]

Bel-7402 cells line were used in this work from the American type culture collection (ATCC). Cells were cultured in cell culture dishes with 10% fetal bovine serum and 90% 1640 medium at 37°C with 5% CO_2 (standard culture conditions) for 24 hours to keep the cells from being suspended. Until the density of cells reached 2×10^6 cells per mL. Subsequently, the cells were split into a 35 mm petri dish, the density of cells should keep around 3×10^5 cells in each dish, then cells were incubated for 24 h under standard culture conditions. After culturing overnight, The staining solutions for cell staining were prepared by adding an aliquot of Lys-CzFP (10 μM) and Lyso-Tracker Red (10 μM) into the culture medium. And it incubated for 30 min under standard culture conditions. After that, the imaging sample was washed with PBS (pH 7.4) three times to remove the free probe and Lyso-Tracker Red. Then, the cells were imaged using confocal laser scanning microscopy. (FLUOVIEW FV3000. OLYMPUS)

Tracking viscosity changes experiment[5]

Bel-7402 cells seeded in a 35 mm petri dish with a glass cover slide. After culturing overnight, a specific concentration of the Lys-CzFP (10 μM) solution prepared in DMSO was added to the above petri dish. And it was incubated with the cells for 30 minutes under standard culture conditions. Then, dexamethasone (20 μM)

was added and incubated for 60 min. Before imaging, the cells were washed three times with PBS solution (pH 7.4) to remove the free probe. Recording real-time living cells images every 20 min under confocal laser scanning microscopy. (FLUOVIEW FV3000. OLYMPUS)

Nuclear staining experiment[6]

Hela cells line were used in this work from the American type culture collection (ATCC). Hela cells seeded in a 35 mm petri dish with a glass cover slide. After culturing overnight, a specific concentration of the Lys-CzFP (10 μM) solution prepared in DMSO was added to the above petri dish. And it was incubated with the cells for 30 minutes under standard culture conditions. Then, DAPI (10 μM) was added and incubated for 40 min. Before imaging, the cells were washed three times with PBS solution (pH 7.4) to remove the free probe and DAPI. Recording real-time living cells images under confocal laser scanning microscopy. (FLUOVIEW FV3000. OLYMPUS).

The photostability experiment

Hela cells seeded in a 35 mm petri dish with a glass cover slide. After culturing overnight, a specific concentration of the Lys-CzFP (10 μM) solution prepared in DMSO was added to the above petri dish. And it was incubated with the cells for 24 h under standard culture conditions. Before imaging, the cells were washed three times with PBS solution (pH 7.4) to remove the free probe. Recording real-time living cells images every 8 h under confocal laser scanning microscopy. (FLUOVIEW FV3000. OLYMPUS).

Fig. S1 ^{1}H NMR (600MHz, $CDCl_3$) spectrum of Compound M_1.

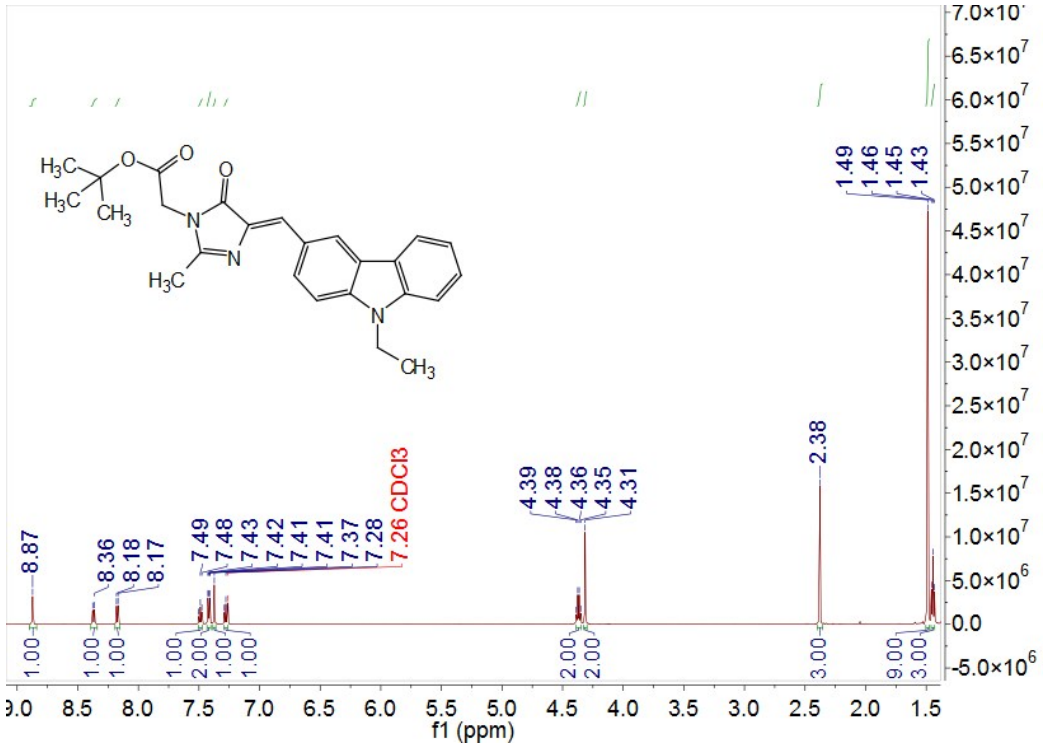


Fig. S2 ^{1}H NMR (600MHz, DMSO-d_6) spectrum of Compound M_2

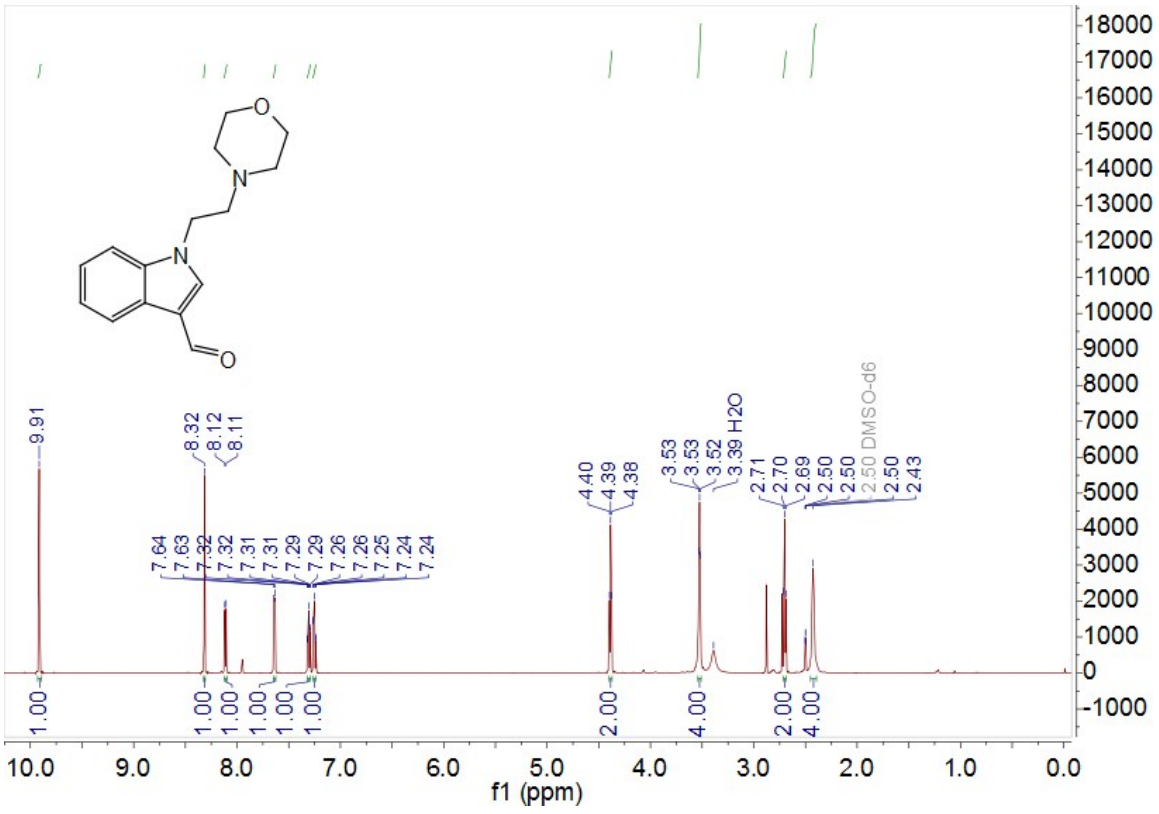


Fig. S3 ^{1}H NMR (300MHz, DMSO-d_6) spectrum of Compound Lys-CzFP.

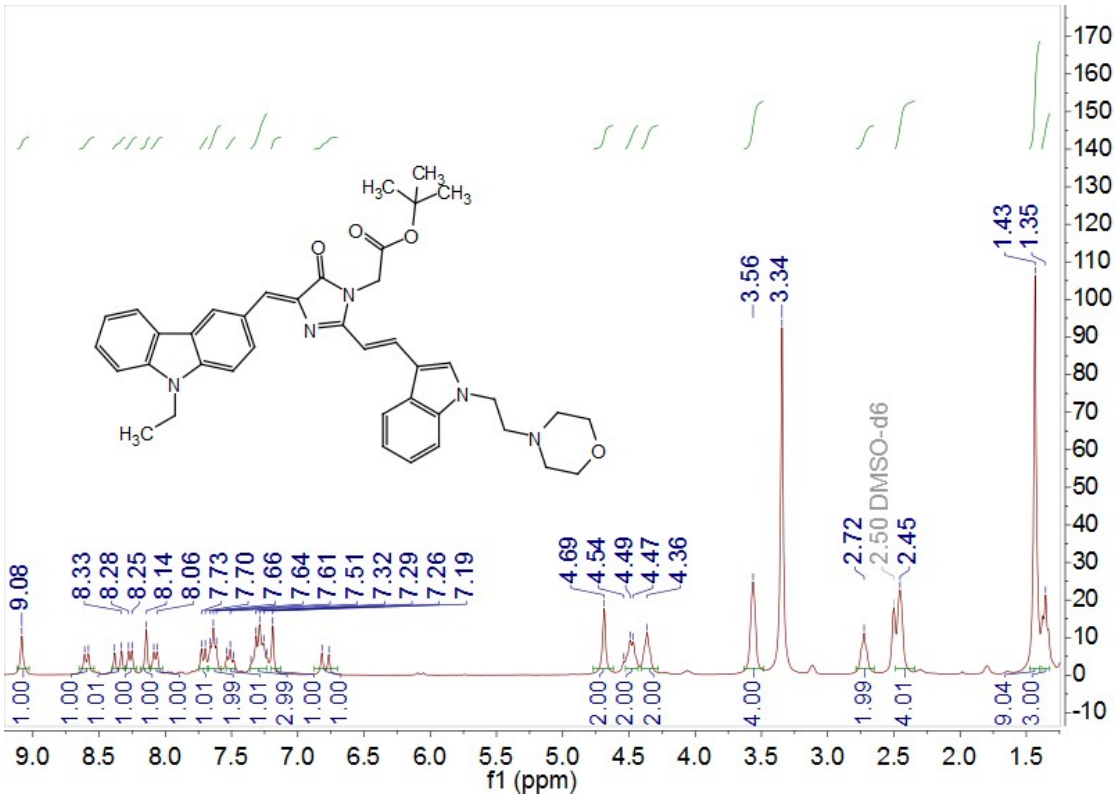


Fig. S4 ^{13}C NMR (75MHz, DMSO-d_6) spectrum of Compound Lys-CzFP.

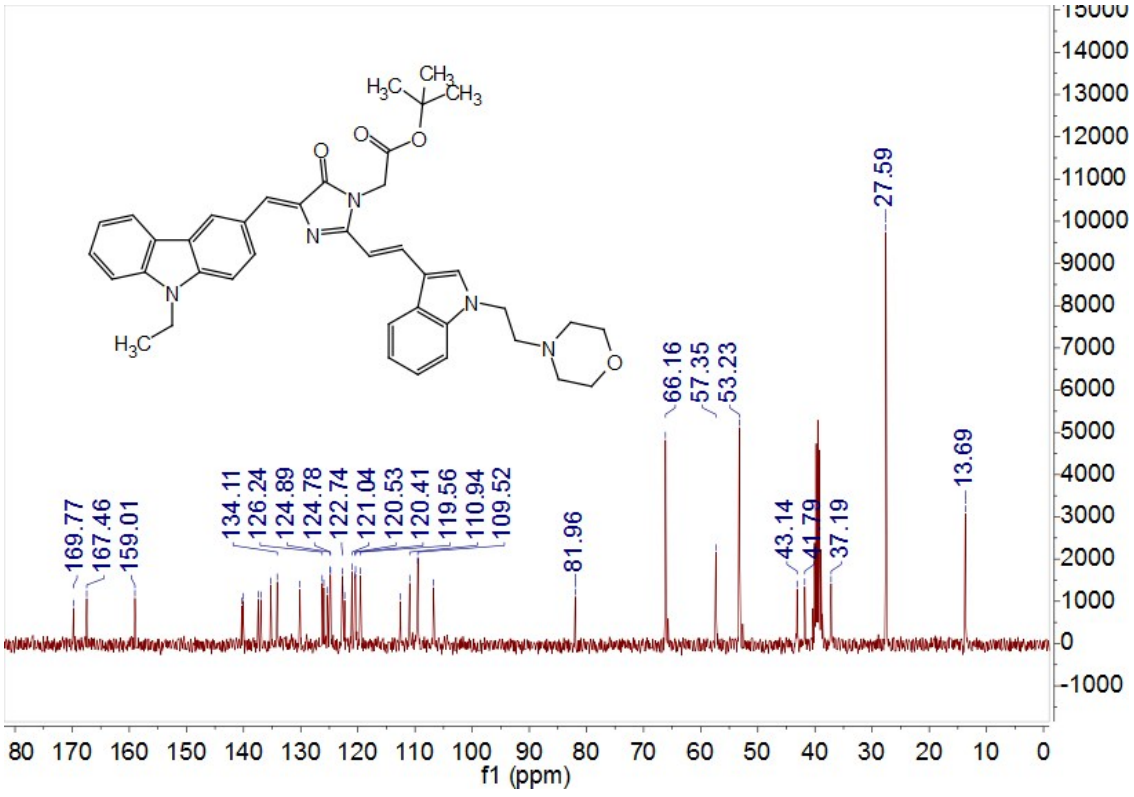


Fig. S5 HRMS of Compound Lys-CzFP.

Sample Name	ZX-target	**Position**	Vial 8	**Instrument Name**	Instrument 1
User Name		**Inj Vol**	1	**InjPosition**	
Sample Type	Sample	**IRM Calibration Status**	Success	**Data Filename**	ZX-target.d
ACQ Method	method-POSI.m	**Comment**		**Acquired Time**	5/17/2019 9:44:41 AM (UTC+08:00)

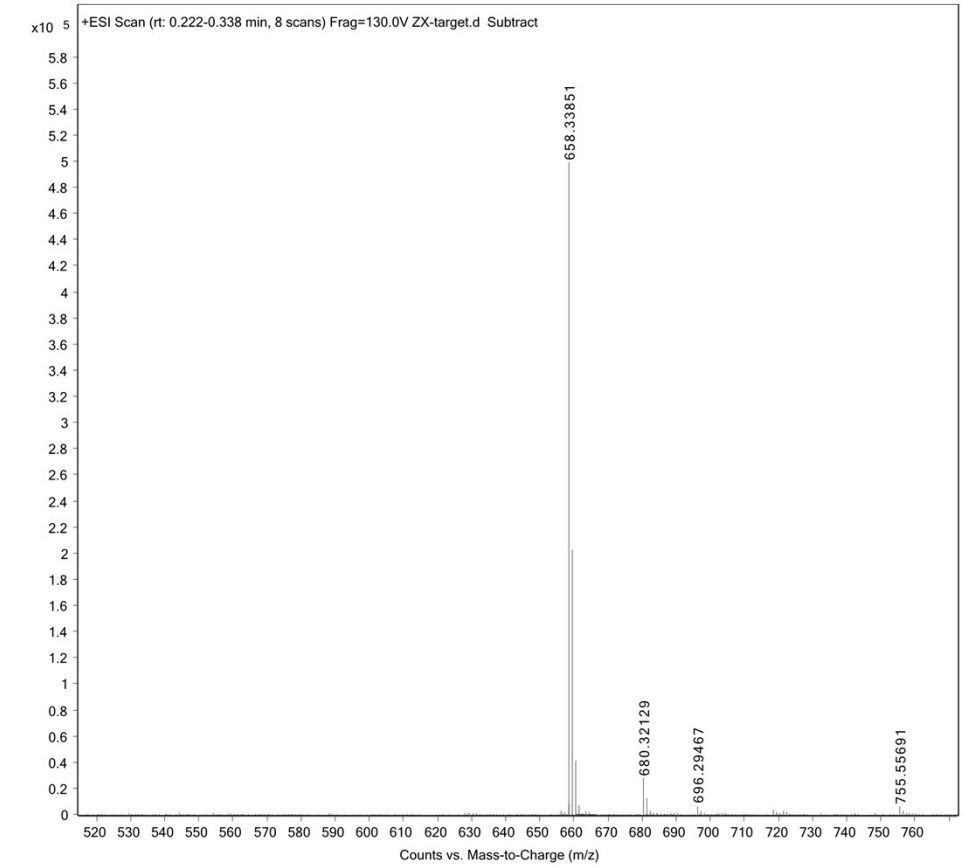


Table S1 Previous work for detection of viscosity

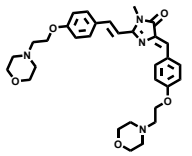
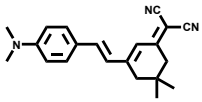
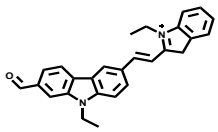
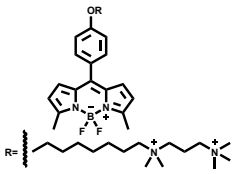
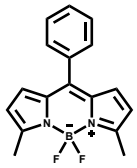
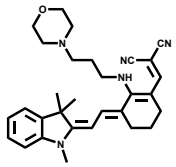
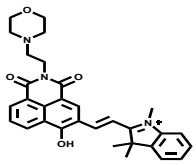
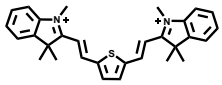
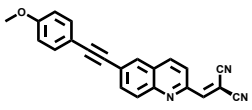
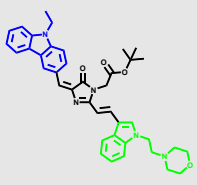
Structures	Response fold	$\lambda_{abs}^{max}/\lambda_{em}^{max}$ (nm)	Test systems	Δλ(nm) φ_F τ(ns)	References/Imaging application
	10	430/515	PBS-Glycerol	~85 - -	Journal of Materials Chemistry B, 2018, 6, 6592-6598 / MCF-7 cells[7]
	25	500/700	PBS-Glycerol	~200 - 0.10 -0.18	Chem Commun, 2016, 52, 13695-13698 / HeLa cells[6]
	40	480/570	Water-Glycerol	~90 0.04-0.27 -	Tetrahedron Letters, 2018, 59, 4540-4544 / HeLa cell[8]
	39	485/510	Methanol–Glycerol	~25 Increased 0.23-2.2	*Chem. Commun., 2014, 50, 5282--5284 /* SK-OV-3 cells[9]
	5	500/515	Methanol-Glycerol	~15 0.017-0.69 0.16-4.35	Chemistry a European journal, 2019, 25, 10342 – 10349 / -[10]
	33	565/615	PBS-Glycerol	~45 0.012-0.36 -	Chem Commun, 2019, 55, 2688-2691 / SH-SY5Y cells[4]
	50	550/610	Water-Glycerol	~60 - -	Journal of Materials Chemistry B, *2018, 6, 580-585 /* RAW.264.7 cells[11]
	25	525/600	Methanol-Glycerol	~75 - -	*Biosens. Bioelectron, 2016, 86, 885-891 /* HeLa cells[12]
	90	365/470	Water–Glycerol	~105 - Increased	Journal of Materials Chemistry B, 2017, 5,2743-2749 / HeLa cells[13]
	98	482/560	Water–Glycerol Methanol-Glycerol Ethylene glycol-Glycerol	78 0.003-0.253 0.24-1.12	This work / Bel-7402 and HeLa cells

Fig. S6 Viscosity titration in Ethanol /Glycerol mixture

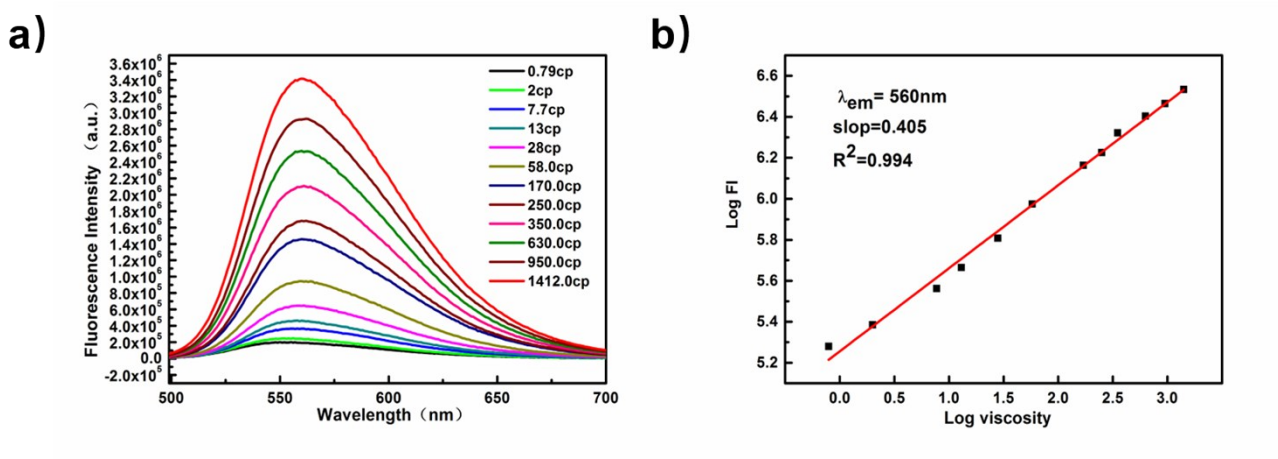


Fig. S6 (a) Fluorescence spectra of Lys-CzFP (10 μM) in different viscosity mixture (Ethanol/Glycerol, viscosity values from 0.79cP to 1412cP, pH 7.4, 20°C, containing 1% DMSO). (b) The linear relation between fluorescence intensity and viscosity.

Fig. S7 Viscosity titration in Ethanediol /Glycerol mixture

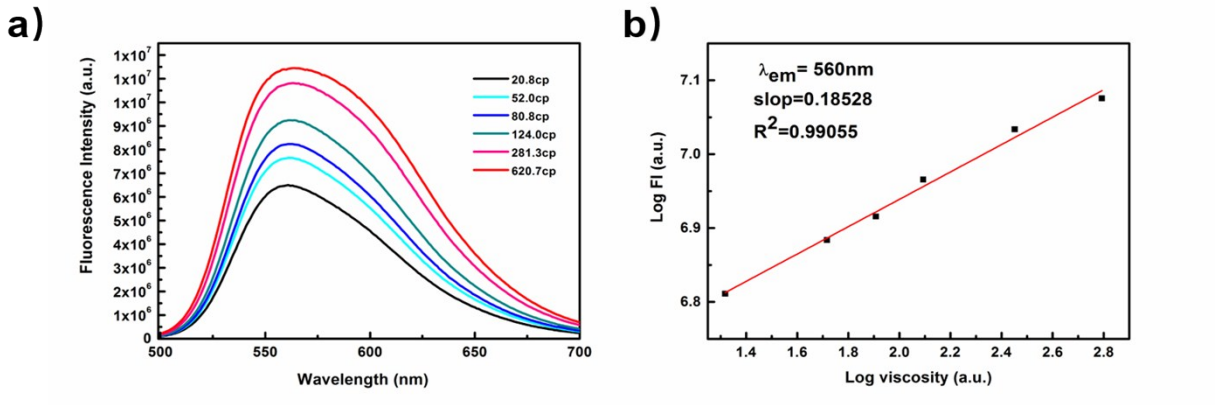


Fig. S7 (a) Fluorescence spectra of Lys-CzFP (10 μM) in different viscosity mixture (Ethanediol/Glycerol, viscosity values from 20.8cP to 620.7 cP, pH 7.4, 20°C, containing 1% DMSO). (b) The linear relation between fluorescence intensity and viscosity.

Fig. S8 UV and fluorescence spectrums of Lys-CzFP in the different solvents.

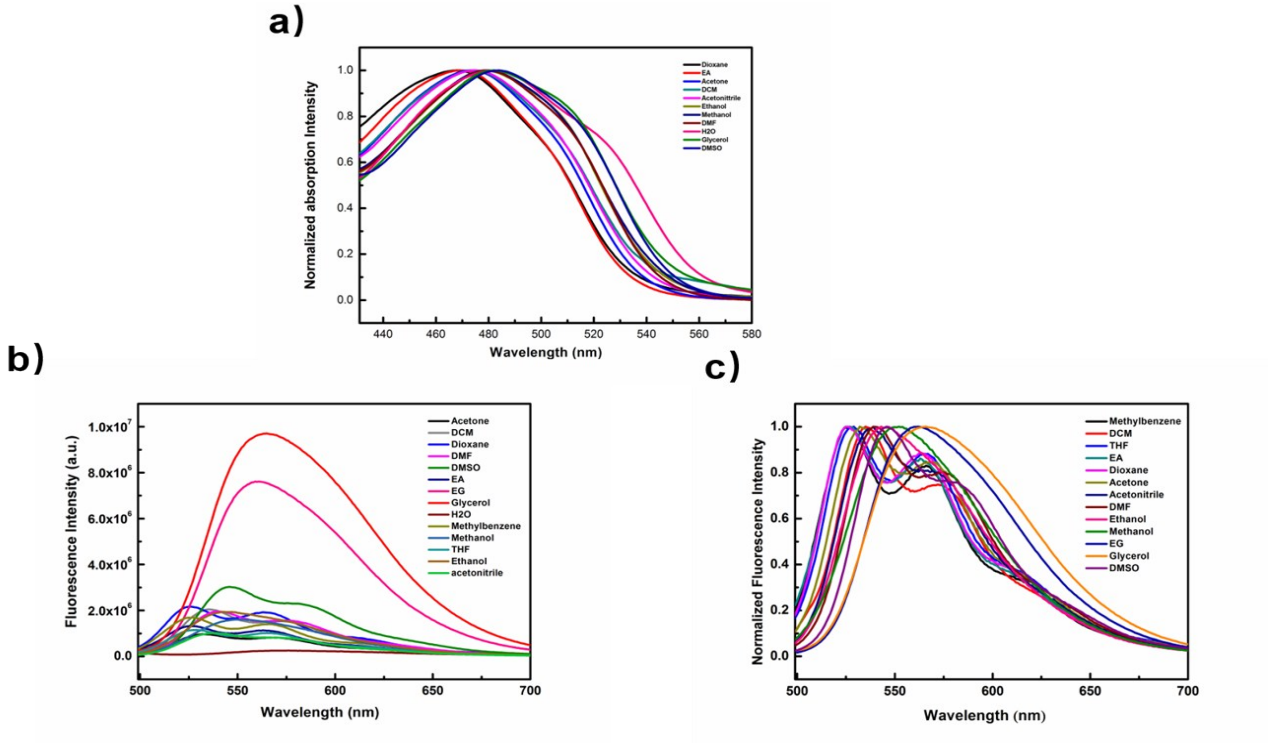


Fig. S8 (a) Normalized absorption intensity. (b) Fluorescence Intensity in various solvents with different polarities. (λ_{ex}=482nm, 20°C, containing 1% DMSO).

We tested the fluorescence spectrum of the probe Lys-CzFP in various solvents. According to Fig .S8a, the UV absorption spectrum presented red-shifted significantly, with the polarity of the solvent increased. The reason could be that ultraviolet absorption of Lys-CzFP concentrated on the electron transition of $\pi \rightarrow \pi^*$. As the polarity of the solution increased, π^* orbit was more stable than π orbit, and the energy level was reduced between π and π^* orbital. Thus, the absorption band presented red-shifted.

According to Fig. S8b, the emission spectrum of Lys-CzFP exhibited a double peak in benign solutions; this could be the LE (Locally excited) peak about and the TICT (Twisted Intramolecular charge transfer) peak. According to Fig. S8c, as the polarity of the solvent increased, the molecular fluorescence spectrum showed a significant redshift, conversely, exhibited a hypsochromic shift. These data of UV absorption spectroscopy and fluorescence emission spectroscopy showed that Lys-CzFP was a typical viscosity molecule.

Fig. S9 Selective and interference experiments

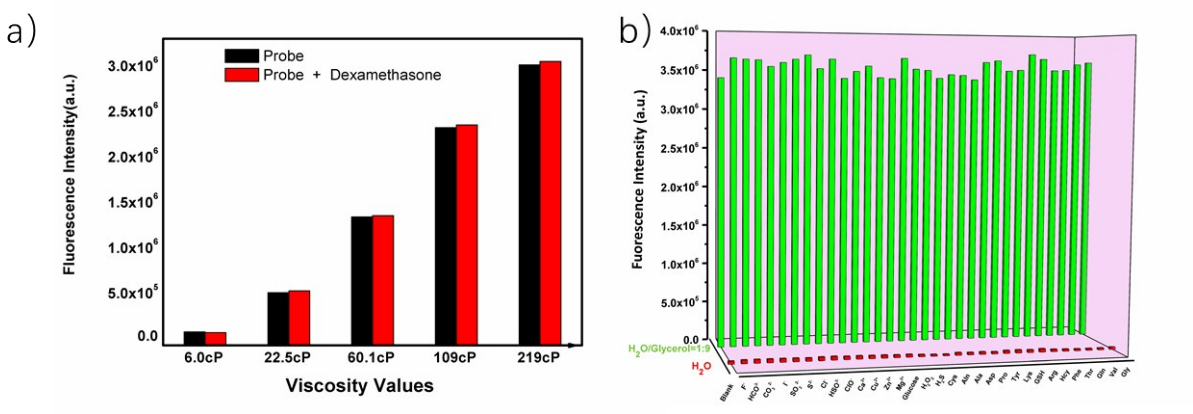


Fig. S9 (a) Maximum fluorescence intensity of Lys-CzFP (10 μM) and Lys-CzFP (10 μM) with 5 eq dexamethasone at various viscosities. 20 °C. (b) The fluorescence responses of Lys-CzFP in water and glycerol (containing 10% water as co-solvent) to various analytes (1 – 32: Blank, F^-, HCO_3^-, CO_3^{2-}, I^-, SO_3^{2-}, S^{2-}, Cl^-, HSO_3^-, ClO^-, Ca^{2+}, Cu^{2+}, Zn^{2+}, Mg^{2+}, Glucose, H_2O_2, H_2S, Cys, Aln, Ala, Asp, Pro, Tyr, Lys, GSH, Arg, Hcy, Phe, Thr, Gln, Val, Gly). (λ_{ex} = 482nm, 20°C, containing 1% DMSO).

Fig. S10 Viscosities titration in various pH aqueous buffer solutions

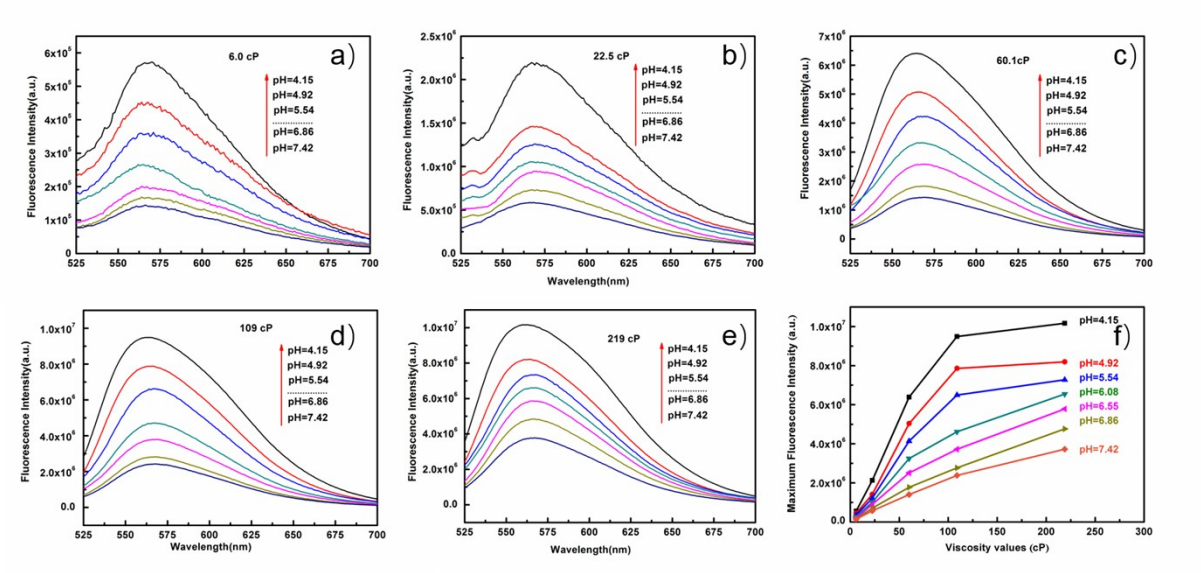


Fig. S10 (a-e) Fluorescence spectrum of the probe Lys-CzFP (10 μM) in different pH aqueous buffer solutions at various viscosities. (a) 6.0 cP; (b) 22.5 cP; (c) 60.1 cP; (d)109 cP; (e) 219cP; (f) Maximum fluorescence intensity in various viscosities at different pH buffer solutions.(λex = 482nm, 20°C, containing 1% DMSO).

Fig. S11 pH titration in various viscosities

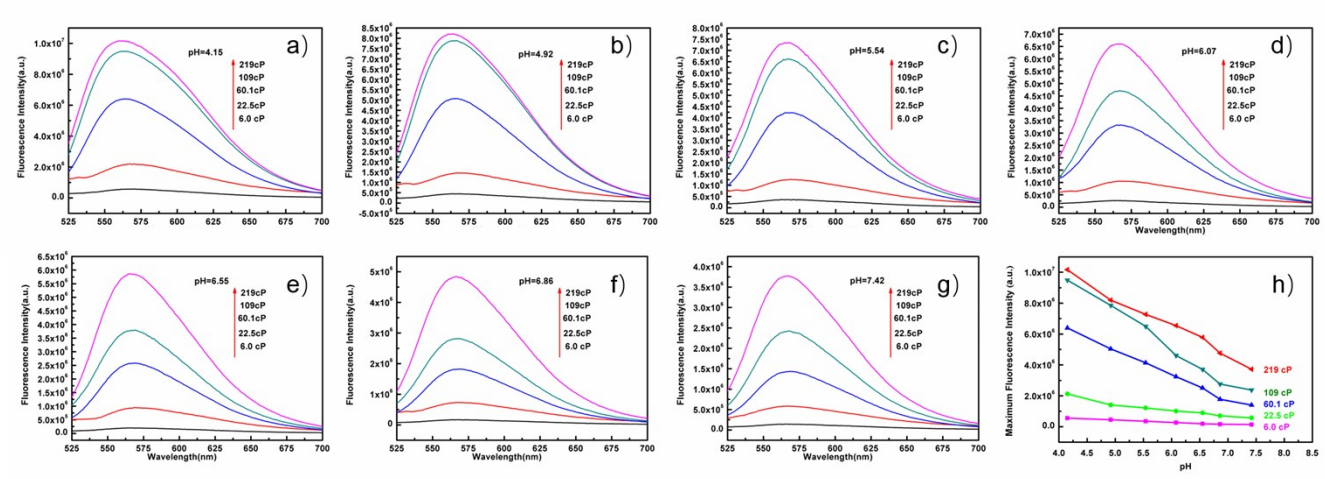


Fig. S11 (a - g) Fluorescence intensity of Lys-CzFP (10 μM) in various viscosities at different pH aqueous buffer solutions. (a) pH = 4.15; (b) pH = 4.92; (c) pH = 5.54; (d) pH = 6.07; (e) pH = 6.55; (f) pH = 6.86; (g) pH = 7.42; (h) Maximum fluorescence intensity in different pH buffer solutions at various viscosities.(λex = 482nm, 20°C, containing 1% DMSO).

Fig. S12 Fluorescence lifetime experiments

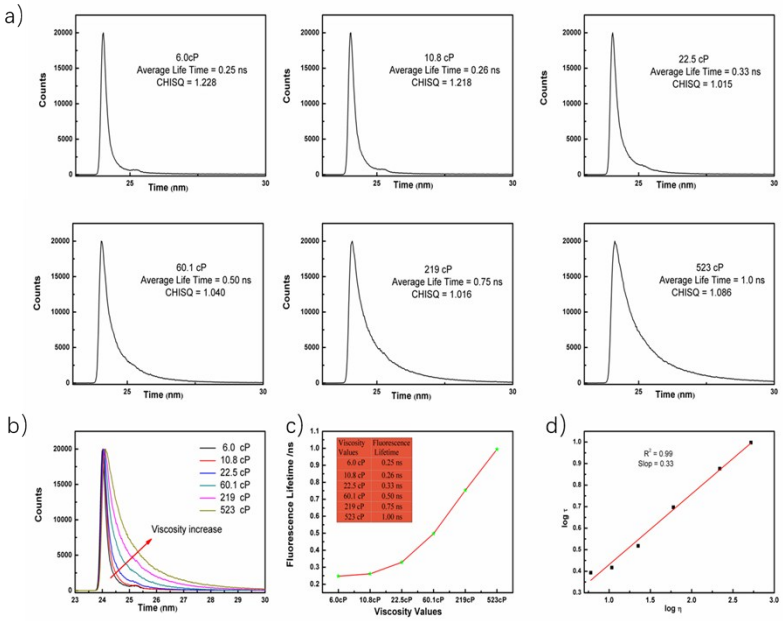


Fig. S12 (a-c) Time-resolved lifetime decay curves of Lys-CzFP (10 μM) in glycerol/water mixed solvents (Ex = 460 nm). (d) Linear relationship between lifetime (τ) and viscosity (η), $R^2 = 0.99$, Slop = 0.33.

Cell experiment

Fig. S13 MTT experiment

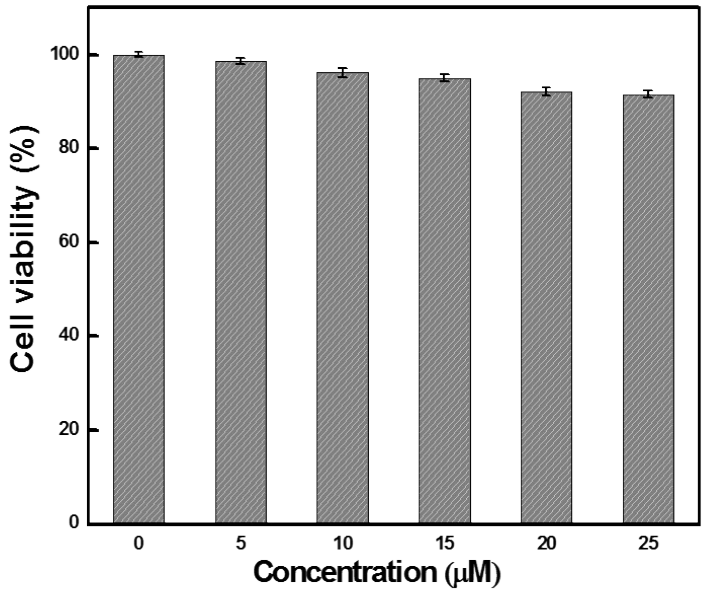


Fig. S13 Cytotoxicity assays of Lys-CzFP at various concentrations for Bel-7402 cells, Error bars represent the standard deviations of 3 trials.

Fig. S14 DAPI experiment

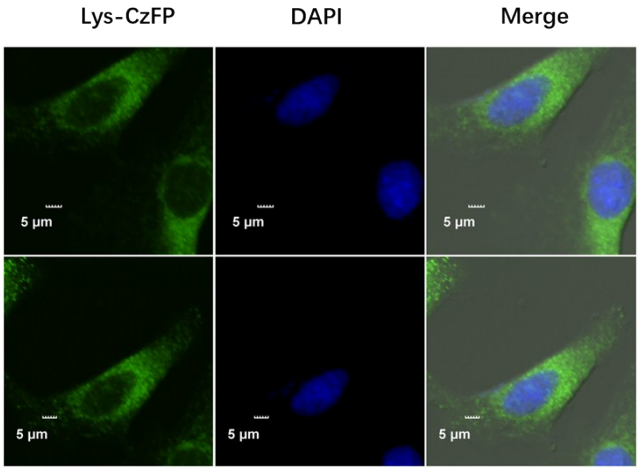


Fig. S14 Confocal fluorescence image of Hela cells co-stained with Lys-CzFP (10 μM) and DAPI (10 μM). Green channel (Ex = 490 nm, Em =561 nm) for Lys-CzFP and Blue channel (Ex = 405 nm, Em = 450 nm) for DAPI, respectively. (25°C) Scale bar is 5 μm.

Fig. S15 The photostability of the probe in living cells

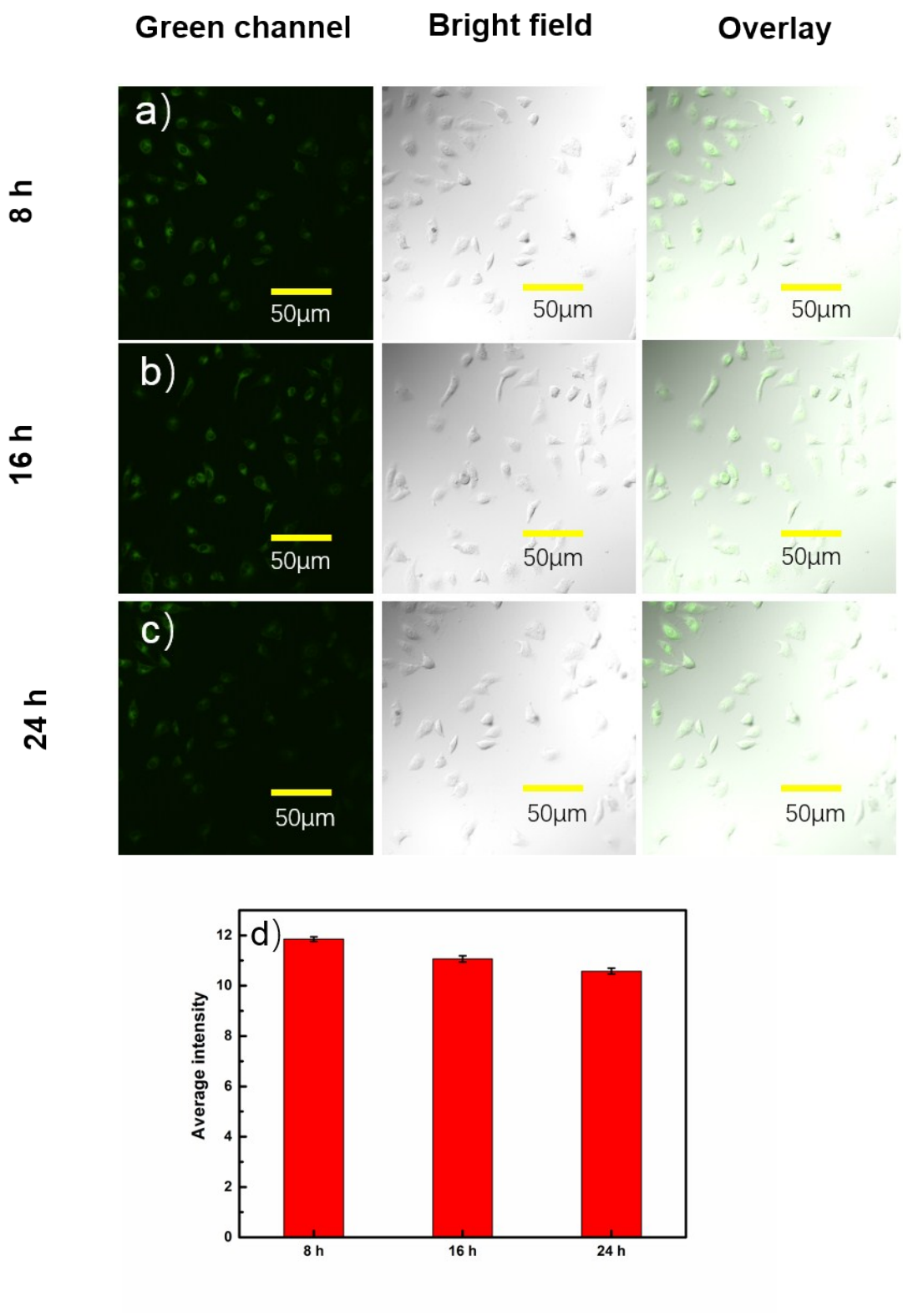


Fig. S15 Confocal fluorescence image of Hela cells with 10 μM probe Lys-CzFP for 8 h, 16 h, 24 h. Green channel (Ex = 490 nm, Em =561 nm). (d) The average intensity of images a-c.

Fig. S16 Real-time living cell images for tracking viscosity changes

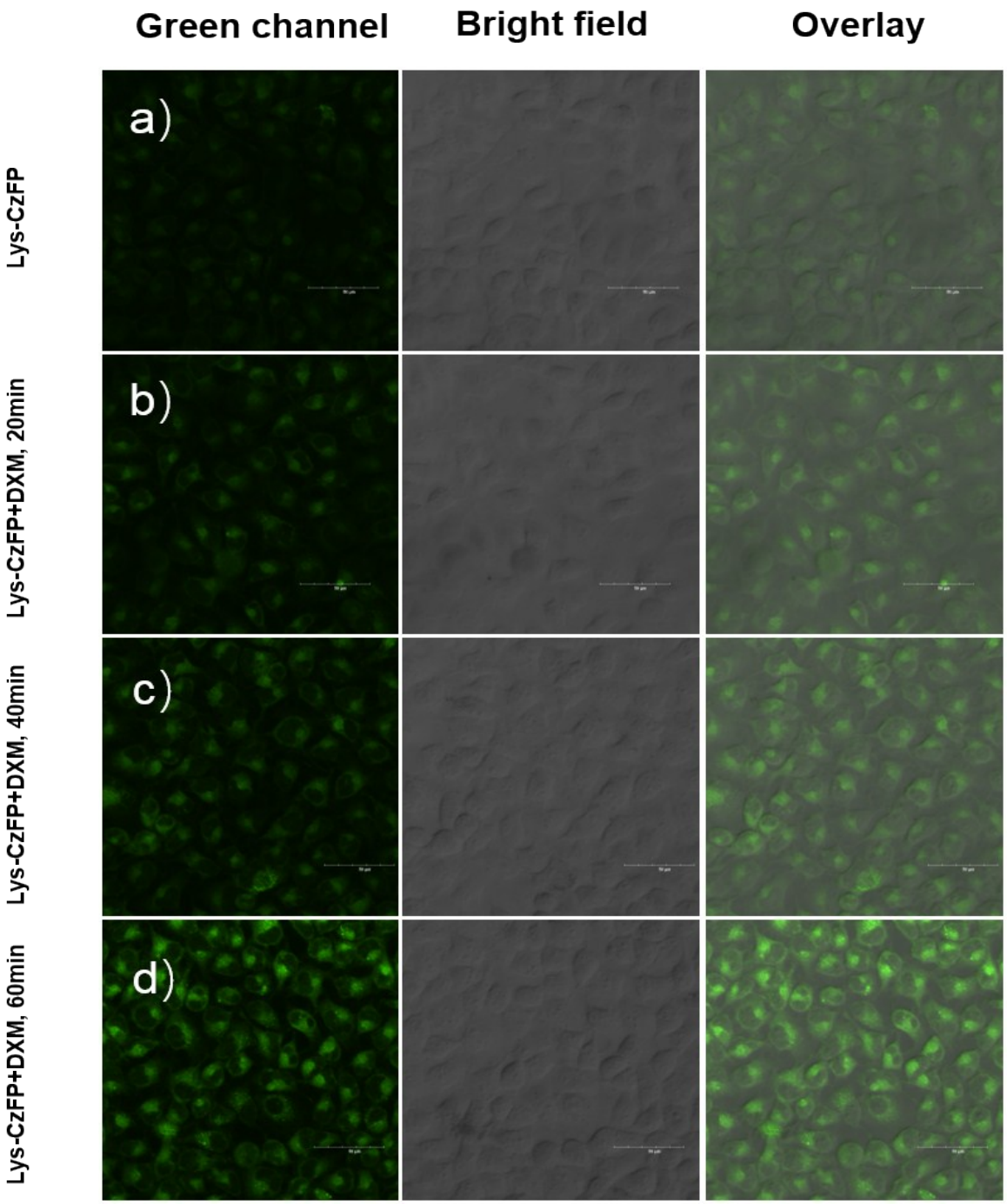


Fig. S16. (a) Confocal fluorescence images of the Bel-7402 cells incubated with 10 μM probe Lys-CzFP for 30 min; (b–d) Confocal fluorescence images of the Bel-7402 cells incubated with 20 μM dexamethasone (DXM) for 60 min. Fluorescence images were taken every 20 min until 60 min later, Scale bar is 50 μm.

Table S2. Viscosity values in different solutions.

V %		Viscosity / cP 20°C
Glycerol	Water	
100%	0%	1412
95%	5%	523
90%	10%	219
85%	15%	109
80%	20%	60.1
75%	25%	35.5
70%	30%	22.5
60%	40%	10.08
50%	50%	6.00
40%	60%	3.72
20%	80%	1.76
0%	100%	1.01

Table S3 The photo-physical data of probe Lys-CzFP in different solvent systems. (Arranged by the polarity of solvent viscosity)

Solvents	Dielectric constant	$\eta^{[a]}$ (cP)	$\lambda_{abs}{}^{max}$ [b] (nm)	$\lambda_{em}{}^{max}$ [c] (nm)	$\Phi_F{}^{[d]}$	$\Delta\lambda^{[e]}$ (nm)	$\varepsilon^{[f]}$ ($M^{-1}cm^{-1}$)	$B^{[g]}$ ($M^{-1}cm^{-1}$)
Dioxane	**2.2**	**1.54**	**468**	**526**	**0.012**	**58**	**20482**	**246**
Ethanol	**2.5**	**1.07**	**478**	**534**	**0.012**	**56**	**27352**	**328**
EA	**6.02**	**0.45**	**469**	**525**	**0.009**	**56**	**22894**	**206**
DCM	**9.1**	**0.43**	**473**	**535**	**0.013**	**62**	**24185**	**314**
Acetone	**20.7**	**0.32**	**473**	**532**	**0.006**	**59**	**20720**	**124**
Methanol	**32.6**	**0.59**	**479**	**552**	**0.025**	**73**	**22031**	**551**
DMF	**36.7**	**0.80**	**480**	**539**	**0.013**	**59**	**24317**	**316**
MeCN	**37.5**	**0.37**	**474**	**539**	**0.007**	**66**	**22278**	**156**
Glycerol	**45.8**	**1412**	**482**	**560**	**0.253**	**78**	**35300**	**8931**
DMSO	**48.9**	**2.24**	**484**	**546**	**0.026**	**62**	**24360**	**633**

Table S4 The photo-physical data of probe Lys-CzFP in different solvent systems. (Arranged by the value of solvent viscosity)

Solvents	Dielectric constant	$\eta^{[a]}$ (cP)	λ_{abs}^{max} [b] (nm)	λ_{em}^{max} [c] (nm)	Φ_F[d]	$\Delta\lambda$[e] (nm)	ε[f] ($M^{-1}cm^{-1}$)	B[g] ($M^{-1}cm^{-1}$)
Acetone	**20.7**	**0.32**	**473**	**532**	**0.006**	**59**	**20720**	**124**
MeCN	**37.5**	**0.37**	**474**	**539**	**0.007**	**66**	**22278**	**156**
DCM	**9.1**	**0.43**	**473**	**535**	**0.013**	**62**	**24185**	**314**
EA	**6.02**	**0.45**	**469**	**525**	**0.009**	**56**	**22894**	**206**
Methanol	**32.6**	**0.59**	**479**	**552**	**0.025**	**73**	**22031**	**551**
DMF	**36.7**	**0.8**	**480**	**539**	**0.013**	**59**	**24317**	**316**
Ethanol	**2.5**	**1.07**	**478**	**534**	**0.012**	**56**	**27352**	**328**
Dioxane	**2.2**	**1.54**	**468**	**526**	**0.012**	**58**	**20482**	**246**
DMSO	**48.9**	**2.24**	**484**	**546**	**0.026**	**62**	**24360**	**633**
H_2O/Glycerol = 3/2	**—**	**3.72**	**483**	**567**	**0.054**	**84**	**24850**	**1342**
H_2O/Glycerol = 1/1	**—**	**6.00**	**482**	**567**	**0.059**	**85**	**25324**	**1494**
H_2O/Glycerol = 1/4	**—**	**60.1**	**483**	**564**	**0.116**	**81**	**27308**	**3168**
H_2O/Glycerol = 1/9	**—**	**219**	**482**	**563**	**0.139**	**81**	**29080**	**4042**
Glycerol	**45.8**	**1412**	**482**	**560**	**0.253**	**78**	**35300**	**8931**

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